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Mitigation of ammonia gas from animal house using microalgae

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Mitigation of ammonia gas from animal house using microalgae

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Abstract

We suggest a strong potential of algae to mitigate ammonia gas from animal house and to be used as a high-value animal feed. Ammonia gas emission from animal manure decomposition is a major concern in animal housing operations. Excessive ammonia gas volatilization will affect both animal and worker health and can also cause significant environmental concerns. Current ammonia gas mitigation methods are based on physical, chemical, biological, and dietary treatments, but the costs are high and the performances are not stable. In this project, we proposed an algae-based method for removing ammonia gas generated from animal housing operations while producing a biomass with high protein content which can be potentially used as high-value animal feed products. The green algae *Scenedesmus dimorphus* was used for evaluating its ability to mitigate ammonia gas in a gas-lift photobioreactor under continuous operational mode. Different conditions were tested for optimal algal biomass productivity: 0.05, 0.1, 0.2, and 0.3 day^{-1} of dilution rate; 17, 42, 60, and 72 ppm of ammonia gas concentration in inlet air; and pH 5, 6, 7, and 8. The nitrogen mass balance was calculated for each case and results showed that as high as 98.6 % of nitrogen was assimilated by algae biomass at optimal condition (60ppm, pH 7, and 0.1 day^{-1} of dilution rate). The amino acid profile of the biomass was also analyzed in application of algae as a source of animal feed. This experiment implies two benefits. One is economic benefit, i.e., cost down ammonia gas removal and algae growth, the other is a new algae market: animal feed.

Chapter 1. Introduction

1.1. Project description

With an awareness of seriousness of ammonia gas emission from animal house, this project was designed to explore the possibility of algal photobioreactor to abate ammonia gas and understand how the ammonia gas is removed inside the reactor. In order to maximize the algae biomass production and the amount of ammonia gas reduction, the optimal growth condition was investigated. The ammonia gas removal performance was compared between the growth conditions and with the previous research: mitigating ammonium using algae, as covered broadly in Chapter 4; and mitigating ammonia gas using other methods, as introduced in section 2.4 in Chapter 2. The nitrogen mass balance was assessed to see the ammonia gas fate. As the limiting nutrient in this project was nitrogen, the amino acid profile was assessed as a potential source of an animal feed.

1.2. Thesis organization

In Chapter 2, literature was reviewed to raise awareness of the severity of ammonia gas from animal house, to present principles of ammonia gas generation and ventilation, and to introduce ongoing mitigation methods. In Chapter 3, materials and methods of present study were introduced to show how the algae cells were cultured, how the photobioreactor was built and operated, and how the analyses have conducted. In Chapter 4, the data from each algae growth condition (dilution rate, ammonia concentration in the inlet gas, and medium pH) were compared in terms of cell density, cell productivity, ammonia gas removal performance parameters, and nitrogen mass balance. The amino acid profile of algae grown at the optimal condition was also characterized. In Chapter 5, general conclusion was arrived and future work was suggested.

Chapter 2. Background of ammonia gas generation and emission from animal house and mitigation methods

2.1. Impact of ammonia gas on the environment

Ammonia (NH_3) is a corrosive, colorless gas with a very distinct odor. In the United States, the largest ammonia emission source is livestock operations for production of milk, meat, and eggs [1]. Ammonia gas volatilization from animal houses not only impairs the manure value as fertilizer due to N loss, but also causes considerable environmental and health concerns. Various studies have shown that high ammonia concentration can cause the following negative consequences:

(1) Diseases

The high ammonia concentration inside the animal house will affect the health of animals as well as workers. Several review papers have been published related to the health problems caused by elevated ammonia concentration from animal house including respiratory and retinal diseases, reduced respiratory rate, degrade of egg quality, and retarded animal growth ([2],[3]).

(2) Eutrophication of surface water bodies

Eutrophication occurs when there is excessive amount of nitrogen in the water or soil because nitrogen is one of the limiting factors for algal growth. The overabundance of algae will cloud the water and eventually unbalance the ecosystems.

(3) Soil and water contamination by acidification and leaching

Ammonia reacts with acidic atmospheric species, such as nitrate (NO_3^-) and sulphate (SO_4^{2-}) to form aerosols and is redistributed to land and water [4]. After leaching by rainwater ammonium sulfate reaches to the soil or water and the ammonia is nitrified to nitric acid (HNO_3) and acidification occurs [5].

$$(NH_4)_2SO_4+4O_2 \rightarrow 2HNO_3+H_2SO_4+2H_2O \quad \cdots\cdots\cdots\cdots \text{Equation 2.1}$$

The N uptake by plants is rather low so the leached out ammonia-N disturb the mineral balance of the soil and contaminate groundwater and drinking water [6].

(4) Noxious effect on vegetation or ecosystem

Ammonia can also be directly absorbed by vegetation surface [5]. The introduction of nitrogen surplus to ecosystems might disturb the ecosystem and change biodiversity. The possible adverse effects of ammonia on plants such as foliar injury, growth and productivity alterations, and change in responses to insect pests and pathogens may reshape the biodiversity of the entire ecosystem [7].

(5) Adverse climate effect

Ammonia gas itself is not a greenhouse gas, but it participates in nitrous oxide (N_2O) generation during oxidization to nitrite [8]. Also, ammonia gas reduces air quality by the formation of particulate matter of diameter 2.5 or less.

2.2. Animal house operations

Prior to World War II, the majority of animals were reared in backyard flocks with natural ventilation. With dramatic expansion of animal research and meat industry, modern livestock production occurs primarily in confined buildings in order to protect the animals welfare from outside environment and to increase productivity [9]. While the configurations of animal housing systems vary widely, the animal manure management practices are either through land application or storage inside a pit underneath a floor. Typically, land application of animal manure emits more ammonia but occurs during short period of time and the management is adjustable to minimize ammonia volatilization. On the other hand, manure stored in a pit is accumulated continuously and removed less frequently, creating a favorable environment for degradation of organic nitrogen in the manure into ammonia. To reduce ammonia emission, this manure management practice is gaining more research attention [10].

Here the term ‘manure’ contains various forms: feces or slurry in the pit and the mixture of animal wastes on the floor. The animal wastes mixture includes urine (contains urea for mammals, uric acid for birds), feces (contains urea or uric acid, ammonia, and undigested protein), bedding materials (straw, sunflower hulls, wood shaving), washed water, and dust. Those highly organic materials are a good nutrient source for urease-producing bacteria that are abundant on a floor of animal houses [9]. Urea and uric acid hydrolyzes rapidly to form ammonia which will emit soon after excretion, often within a few days. The formation of ammonia from complex organic nitrogen in feces occurs slowly within months or years, but will continue with the microbial breakdown of manure [1].

2.3. Mechanisms of ammonia gas production and emission

Ammonia gas formation and volatilization from animal houses depends on several factors related to animals (e.g. diet and animal activity), animal wastes (e.g. moisture content, pH, temperature, and surface area), environment (e.g. indoor and outdoor temperature, ventilation flow, and air velocity over the manure surface), and other site-specific factors (e.g. type of bedding materials) [11].

When animals are fed high protein feed, the surplus nitrogen is not metabolized and excreted mostly via the urine. A small amount of urea enters the large intestine from the blood and becomes incorporated into bacterial protein that then is excreted via the feces. Undigested and thus unabsorbed amino acids are excreted into the feces. The biochemical processes of urea or uric acid degradation into ammonia can be simplified as follows [12, 13]:

$$\text{Urea } (CO(NH_2)_2)+3H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^- + OH^- \quad \cdots\cdots\cdots\cdots\cdots \text{Equation 2.2}$$

$$\text{Uric acid } (C_5H_4O_3N_4)+1.5O_2+4H_2O \xrightarrow{\text{Uricase}} \rightarrow\rightarrow \xrightarrow{\text{Urease}} 5CO_2+4NH_3 \cdots\cdots\cdots\cdots\cdots \text{Equation 2.3}$$

The urease and uricase are bacteria originated enzyme and their activities are affected by temperature, pH and water activity. Urease activity shows exponential increase relative to temperature from 10℃ up to 60℃ and has optimum pH ranging from 6 to 9. Uricase has optimum temperature of 45℃ and stable pH range from 5.5 to 10.0 [14]. The animal manure natural pH is usually between 6.8 and 7.4, therefore the optimal conditions for hydrolysis are generally met in animal housing system [12]. Figures 2.1 and 2.2 show the relative effects of temperature and pH, respectively on degradation rate.

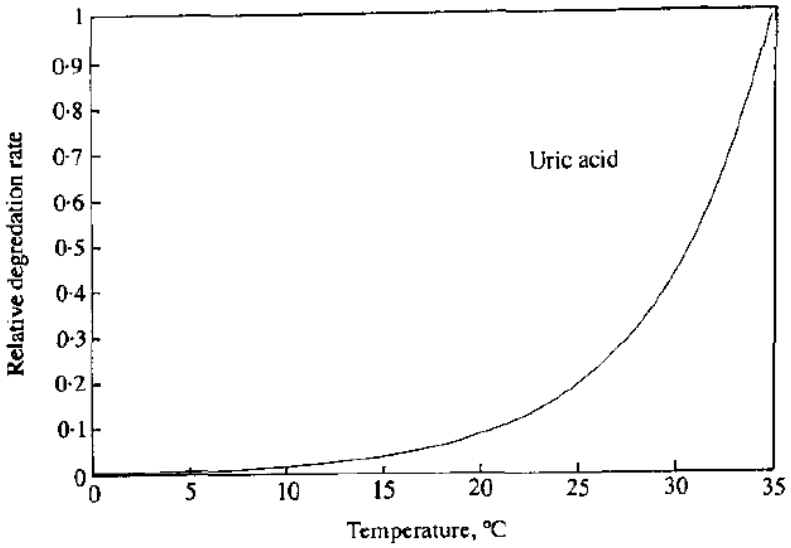

Figure 2. 1 Effect of temperature on the uric acid degradation (adapted from [13])

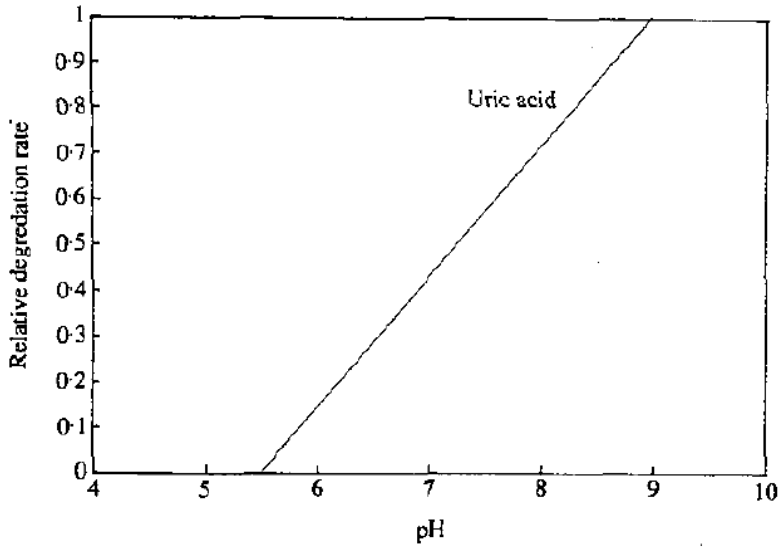

Figure 2. 2 Effect of pH on the uric acid degradation (adapted from [13])

Figure 2.3 shows the litter moisture content effect on ammonia release. It shows that microbial growth is optimal between 40% and 60% moisture content. In practice, the moisture in the animal litter ranges from 20 and 40%, thus, an increase in the moisture enhances ammonia formation [13].

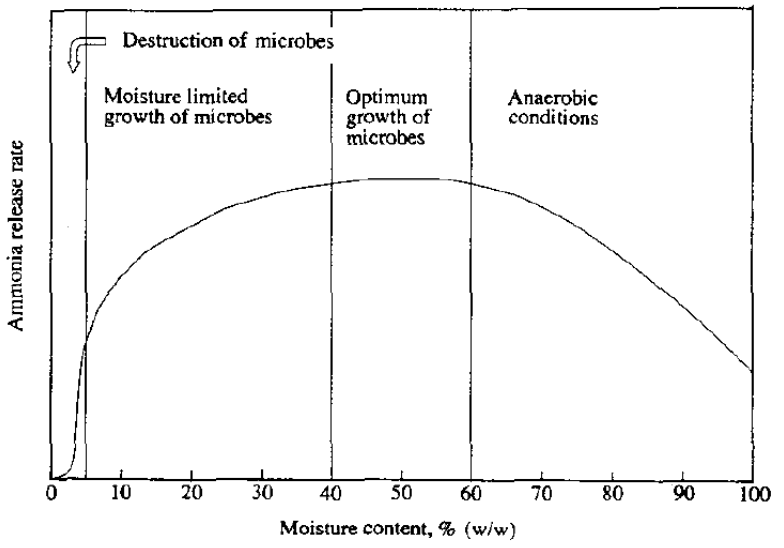

Figure 2. 3 Ammonia release rate dependence on litter moisture content (adapted from [13])

After ammonia formation, two equilibriums determine the ammonia dissociation, namely: ammonium-ammonia equilibrium (Equation 2.4) and ammonia liquid-gas phase equilibrium (Equation 2. 5). In liquid phase, total NH_3-N is in a state of equilibrium between ionized ammonium (NH_4^+) and un-ionized ammonia (NH_3) [12].

$$NH_4^+ (l) \leftrightarrow NH_3(l) + H^+, k_a = \frac{[NH_3][H_3O^+]}{[NH_4^+]}, \mathrm{p}k_a = 9.24\ (25℃) \quad \cdots\cdots\cdots\cdots\cdots \text{Equation 2.4.}$$

Figure 2.4 is derived from Equation 2.4 and it shows the nitrogen species change along with different pH. The dominant N species exist as NH_4^+ at low pH and NH_3 at high pH. The two species exist at the same amount when pk_a is equals to pH. The equilibrium is also affected by temperature. For example, higher temperature results in lower pk_a, so the equilibrium favors ammonia at a higher temperature.

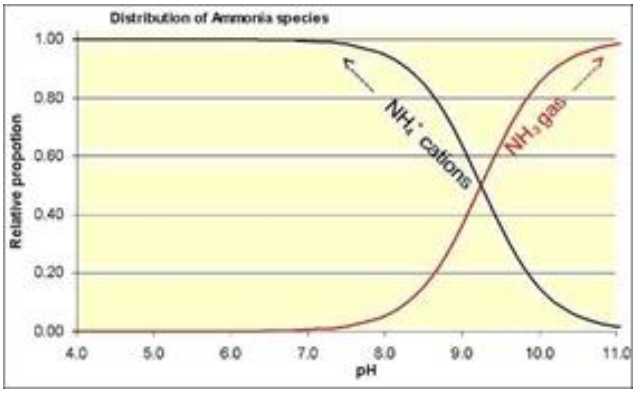

Figure 2. 4 log C-pH diagram for ammonia (adapted from [15])

The ammonia liquid-gas phase equilibrium can be expressed by following:

$$NH_3(l) \leftrightarrow NH_3\ (g) \quad \cdots\cdots\cdots\cdots\cdots \text{Equation 2.5.}$$

The above balance follows Henry's law, so high partial pressure of ammonia in liquid phase results in more ammonia gas concentration. Because the partial pressure increases with the temperature raise, the higher temperature leads to more ammonia gas.

After ammonia gas dissociated from ammonia in liquid phase, the ammonia volatilization to the air follows the mass flux, expressed in Equation 2.6 [13].

$$NH_3(g, manure) \leftrightarrow NH_3\ (g, air) \quad \cdots\cdots\cdots\cdots\cdots\text{Equation 2.6.}$$

The volatilization rate is the product of the difference in partial pressure between the two media (driving force) and a mass transfer coefficient. The partial pressure increase with higher temperature; and the mass transfer coefficients rise with increasing air velocity at the boundary layer and emitting surface area [12].

2.4. Current NH_3 removal methods

The current practices of curbing ammonia emission can be classified as mitigation at ammonia generation phases (i.e., the phase of feces production, degradation, volatilization, ventilation) or end-of-pipe treatment [13, 16].

The abatement methods are categorized by the characteristics of the technologies although some technologies overlap more than one category.

(1) Dietary methods

By adjusting animal diet or feed conversion ability, the nitrogen excretion in feces can be decreased or the urinary pH can be reduced. Numerous research data have proven these effects [12, 17]. However, this is limited by high cost of implementation and N-excretion is not totally prevented by this method [12, 13].

(2) Physical methods

Housing and manure management systems can be designed to prevent ammonia volatilization. Frequent litter changing [18], frequent removal of manure from building (belt houses), and urine-feces separation (floor system) will reduce urine contact with feces and

increase dry matter in manure so that the bacterial activity is inhibited and the degradation rate is minimized. Also, animal house can be designed to control the mass flux, such as reducing surface area of manure exposed to air (battery houses), and reducing air velocity (closed manure storage systems) [13]. It should be noted that, however, reducing ammonia emission using housing and manure management system alone is not an ideal method and sometimes has concerns of animals welfare, economics and technical implementation.

Ammonia adsorption to substances (e.g. zeolite, peat moss, and commercial products) that have high affinity for binding to NH_4^+ ions is another physical method. Those substances are usually more effective on slurries than dry feces [17]. Although significant reductions have been achieved using zeolite and sphagnum peat moss, large quantities of these additives are required so it may increase the amount of manure [13].

(3) Chemical methods

Lowering pH will reduce the enzyme activity and make NH_3-N to exist as ionized form (NH_4^+). This can be achieved by adding acids or acidifying additives (e.g. alum, ferric chloride, and calcium chloride) to the manure. However, there are several limitations of the chemical methods. First, due to heterogeneous nature of the manure, the acid is not always mixed well with the manure. As a consequence, an acid layer will be formed on top of the manure and cause corrosion to the facility. Also, it cannot prevent the dissociation of the ammonia gas from ammonium completely. Second, increased moisture content due to the addition of acid solutions will reduce its feasibility. As shown in Figure 2.3, ammonia formation increases with the increment of moisture content when moisture content is lower than 40 %. Finally, adding acid solution to manure cause undesirable environmental concerns [13]. In addition to the above methods, adding urease inhibitor to manure is another way to reduce ammonia formation. By inhibiting urease activity, the hydrolysis described in Equations 2.2 and 2.3 can be blocked or delayed so the ammonia emission can be reduced. Several researchers have tried different urease inhibitors in lab scale and showed significant reduction of urea hydrolysis [17]. However, in case of full scale animal house application, large amount of additives might bring unknown side effects on the crops or pastures [17].

Acid scrubber is another type of chemical method and can result in an ammonia removal rate ranging from 91 to 99% [16]. The pH in the acid scrubber is usually controlled below 4 by adding acid to the water droplet. A schematic diagram example of an acid scrubber is shown in Figure 2.5.

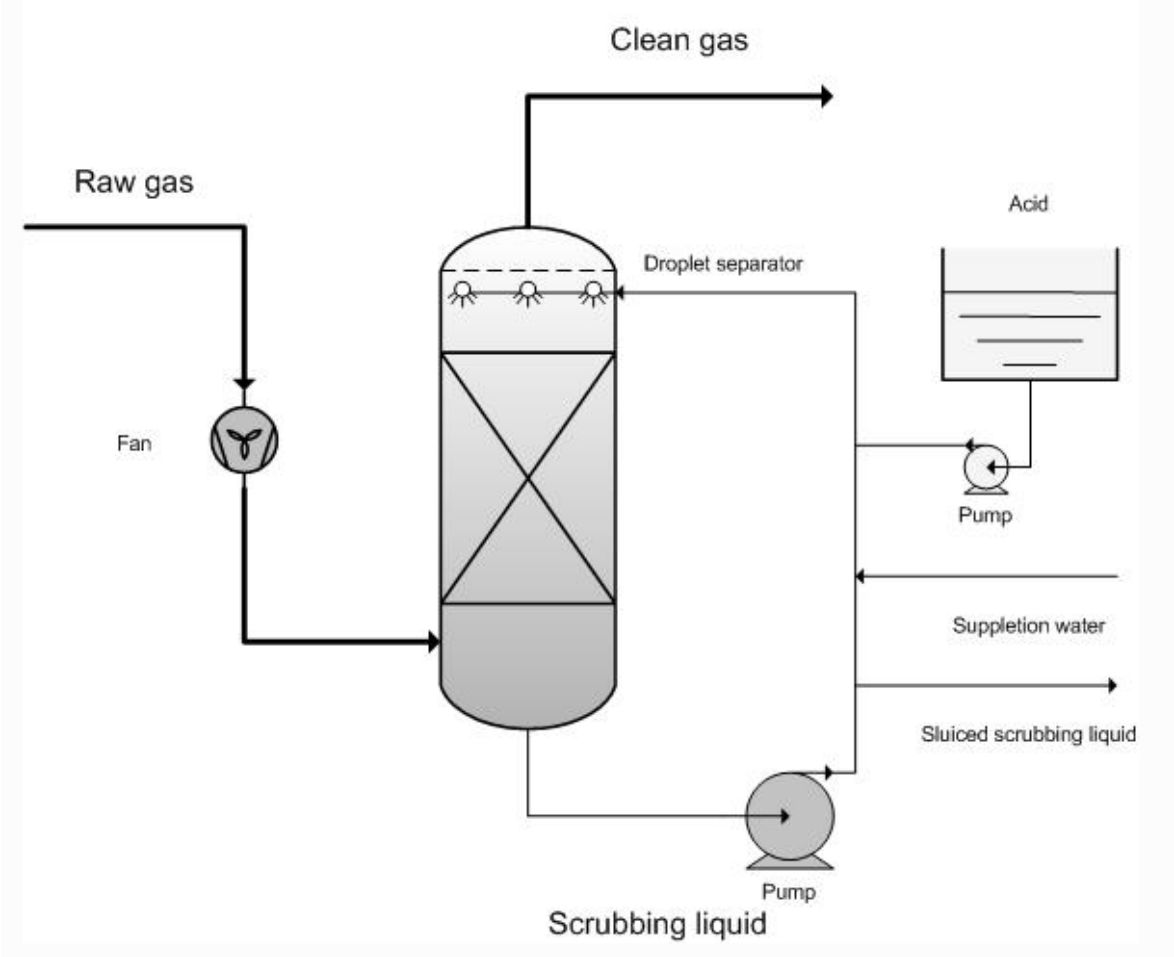

Figure 2. 5 Schematic diagram of acid scrubber for ammonia removal (adapted from [19])

Despite its high performance, the high cost of installation and maintenance of acid scrubber limits its practical applications. It also generates wastewater to prevent unwanted precipitation of ammonium salt in the system [16]. Moreover, only mechanically ventilated houses can use this device and it does not reduce ammonia concentrations inside the house [13].

(4) Biological methods

After ammonia gas volatilized, the ammonia concentration in the outgoing air can be reduced using nitrifying bacterial biofilter or algal nitrogen assimilation.

Among the biological ammonia treatment endeavors, using biofilter has been reported to be an effective method (from 35 to >90% of ammonia removal, [16]). Figure 2.6 shows the schematic design of biofilter. In a biofilter, nitrifying bacteria is inoculated into the packing media while the treated fluid flows through it. Although it is one of the efficient ammonia mitigation tools, the appropriate packing material has not yet been clearly determined and the highest tolerant ammonia concentration to bacteria is relatively low (35 ppm) [20].

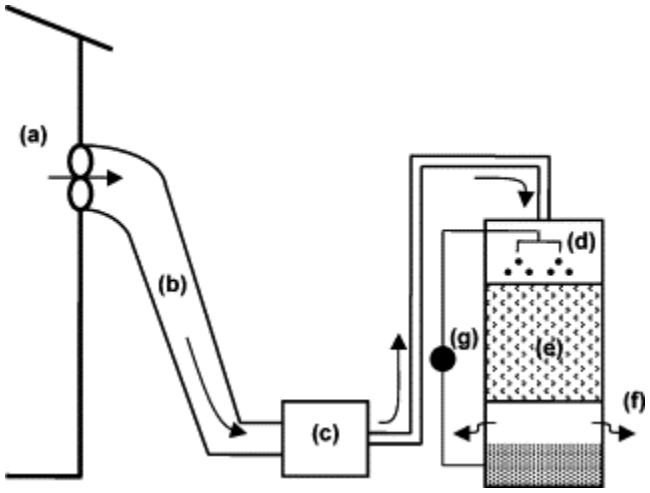

Figure 2. 6 Biofilter system for ammonia removal (adapted from[12]): (a) exhaust fan, (b) air duct, (c) humidifier, (d) splinker zone, (e) packing media, (f) air outlet, (g) pump

Another biological treatment method is to use microalgae as an ammonia scrubber. So far this method has been mainly focused on the dissolved ammonium in a liquid phase in wastewater treatment. This paper is the first report to mitigate ammonia in gas phase using algae.

2.5. Ammonia removal using microalgae

Algae culture system can be used as one of the ‘end-of-pipe’ methods for treatment of ammonia from animal house. This is beneficial in terms of both economics and environment. Because algae need nitrogen for syntheses of nitrogen-content molecules such as proteins and nucleic acids for their growth, providing waste ammonia gas from animal house reduce the costs of the algae cultivation and the produced algae biomass can be further used as an animal feed with high-value proteins [21]. Unlike chemical methods such as strong acids, the algal-ammonia mitigation causes no potential damage to the environment.

Although ammonia in gas phase is remained unexplored, using microalgae for treating ammonia in liquid phase, particularly in wastewater has been intensively investigated. For example, the ammonia effect on *Scenedesmus spp.* has been studied by several researchers ([22-26]) using ammonia in liquid phase and it is well known that the inhibitory effect of ammonia depends on its concentration and the medium pH. The dilution rate effect on the *Scenedesmus spp.* growth when it is used for ammonia removal is also one of the interesting topics because the cell productivity, nutrition competition among different organisms are affected by dilution rate [27].

The biochemical composition of algae is affected by its growth condition, especially the amount of nutrient provided to the algae. For example, more protein will be expressed under higher nitrogen concentration while more lipid or carbohydrate, which is lack of nitrogen, is produced under nitrogen-limiting environment.

The purpose of this study is to examine the *Scenedesmus dimorphus* cell growth and cell productivity in the presence of ammonia gas at different conditions. The fate of nitrogen from ammonia gas and the ammonia gas removal performances were also evaluated. In order to see the potential as an animal feed, the algae biomass was characterized and compared with other cases. This paper will help animal house operators to develop a new ammonia gas trap system and animal feeding system.

Chapter 3. Materials and methods

3.1. Algae strain and medium

The freshwater green algae *Scenedesmus dimorphus* (UTEX 1237) was used. The alga was obtained from the culture collection at University of Texas at Austin and was maintained in agar slant at 5-10 ℃. To prepare seed culture, the cells on agar slant were transferred to 250-mL Erlenmeyer flasks containing 50-mL sterilized modified Bold's Basal Medium ([28, 29]). The modified BBM contains the following chemicals: KH_2PO_4 (175 mg/L), $CaCl_2 \cdot 2H_2O$ (25 mg/L), $MgSO_4 \cdot 7H_2O$ (75 mg/L), $NaNO_3$ (250 mg/L), K_2HPO_4 (75 mg/L), NaCl (25 mg/L), EDTA (50 mg/L), KOH (31 mg/L), $FeSO_4 \cdot 7H_2O$ (4.98 mg/L), H_2SO_4 (1uL), H_3BO_3 (11.42 mg/L), MoO_3 (1.42 mg/L), and trace metal solution (1ml/L): $ZnSO_4 \cdot 7H_2O$ (8.82 g/L), $MnCl_2 \cdot 4H_2O$ (1.44 g/L), $CuSO_4 \cdot 5H_2O$ (1.57 g/L), $Co(NO_3)_2 \cdot 6H_2O$ (0.49 g/L). The pH of the medium was adjusted to 6.8 prior to autoclave at 121℃ for 15 minutes. The flasks were placed in an orbital shaker set at 200 rpm under 25℃ under continuous illumination at 110-120 μmol s^{-1} m^{-2}. The cultures were incubated for 10 days and then transferred to a 5-L (working volume) flat panel photobioreactor to investigate the algal adsorption of the ammonia gas.

3.2. Photobioreactor setup

A flat panel photobioreactor was used in this work. The reactor was made of plexiglass with a dimension of 8.5 ×9.5 ×62 cm (L ×W ×H) and working volume of 5 L (Figure 3.1).

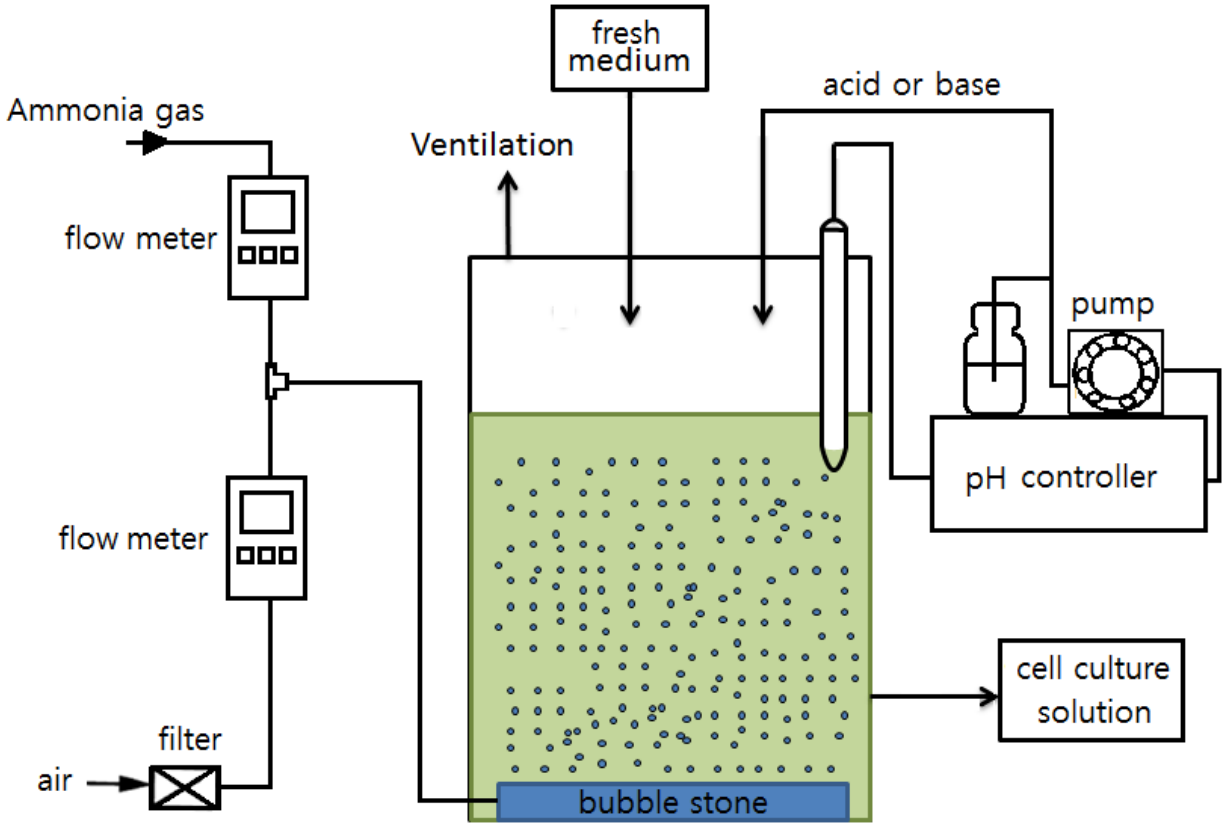

Figure 3. 1 Schematics of the photobioreactor systems for algal culture using ammonia gas as nitrogen source

As shown in Figure 3.1, a gas stream containing compressed air and ammonia gas was introduced to the reactor through a gas diffuser (bubble stone) located at the bottom of the reactor to provide mixing of the liquid. The ammonia gas and air flow rate were respectively regulated by a digital flow meter (MCS series for ammonia gas; MC series for the air, Alicat Scientific, Tucson, AZ) at a pre-set level to achieve the desired ammonia concentration with a total flow rate of 2.774 L/min. The ammonia gas in the inlet gas was ranged from 17-72 ppm to simulate the ammonia gas concentration typically observed in a modern ventilated animal house operation. Before the mixing point of the air and ammonia gas, stainless steel tubing was used to avoid the condensation of the ammonia gas on the tube wall and tubing corrosion, and after the mixing point to the reactor, the mixed gas was connected through Norprene® tubing (4.8mm inner diameter, Cole-Parmer EW-06402-25, Chicago, IL). All the

gas tank and reactor were placed in a lab hood for the safety purpose. The reactor was also equipped with a pH control loop. A pH controller was connected with peristaltic pump (Chemcadet, Cole-Parmer Instrument) to maintain a pre-set pH by adding either hydrochloric acid or sodium hydroxide. In order to avoid pH overshooting or discernible volume change, 0.25M HCl or 0.5M NaOH was used for shifting the operational pH level, while 0.1M HCl or 0.05M NaOH was used for maintaining the pH at a certain level during the operation.

3.3. Photobioreactor operations

3.3.1. Continuous culture

The photobioreactor was run at a batch mode initially for four days then switch to a continuous operation by withdrawing cell suspension from the reactor and feeding the same volume of fresh medium on a daily basis. A medium containing full composition of modified Bold's Basal Medium (BBM) was used in the initial batch cultures. From day 12, $NaNO_3$ in the BBM was replaced with NH_4Cl until day 24 when the cell density reach to a relatively dense level (1.5 g/L). During this first 24 days of operation, no ammonia gas was injected into the reactor. At day 24, ammonia gas was introduced into the reactor at different levels based on the experimental design. At the same time, the nitrogen source in the feed medium (NH_4Cl) was eliminated so the ammonia gas was the only nitrogen source provide to the algal cells in the reactor. Three parameters were studied during the remaining continuous operation: dilution rates, ammonia concentrations in the inlet gas, and medium pH levels.

Samples were taken from the reactor on a daily basis for measuring the cell density. The steady-state under each operation condition was considered to have been established after at least two volume changes (the total volume of liquid flowing through the reactor), with a variation of cell dry weight less than 5% for at least four consecutive days. At the steady state, the withdrawn cell suspension was further centrifuged to collect both the supernatant and cell pellets for future analyses.

3.3.2. Growth conditions

In order to test the effect of ammonia gas to the performance of algae growth and ammonia removal efficiency, different ranges of dilution rate, ammonia gas concentration in the inlet gas, and medium pH in the reactor were studied. As the maximum specific growth rate was determined as 0.32 day^{-1} (see "results and discussion" section), the range of dilution rate was from 0.05 to 0.3 day^{-1}.

The practical chicken house generates ammonia gas of 2-10 ppm in the summer and 10-100 ppm in the winter depending on the types of operation. Also, the Threshold Limit Value (TLV) of ammonia gas is 25 ppm and OSHA permissible Exposure Limit (PEL) is 35 ppm [30]. So 17, 27, 42, 60, and 72 ppm of ammonia gas concentrations were employed to test the algal growth. The pH was diversified around neutral pH (pH 5, 6, 7, and 8) to minimize the use of acid or base.

3.4. Analyses

3.4.1. Cell growth

The algal cell growth was determined by measuring optical density (OD) of the cell suspension at 750nm (OD_{750}) and then converted into cell density (g/L). A spectrophotometer (DU 720, Beckman Coulter, Fullerton, CA) was used for measuring the OD_{750}. A dilution factor 5 was used to ensure the OD value and cell dry weight concentration are in a linear range. After measuring the OD_{750}, it was converted to the cell density (g/L) using a standard curve OD_{750} = 0.9627 g/L + 0.0384 with R^2 of 0.995. The cell productivity (g/L·day) then was calculated by multiplying the cell density (g/L) with the dilution rate (day^{-1}).

3.4.2. Ammonia concentration in the exhausted gas

The ammonia gas outlet was measured by a gas analyzer (BW gas alert Micro5^{TM} electrochemical detector, Honeywell).

3.4.3. Ammonia concentration in the cell culture solution

The algae biomass suspension harvested at the steady state was centrifuged at 3000 rpm for 5 minutes. The algae pellet and the supernatant were separated. The biomass pellets was freeze dried and stored for further characterization.
The supernatant was analyzed by nitrate (Nitrate TNTplus LR, HACH) and ammonia kits (TNT AmVer LR, HACH) using a spectrophotometer (Hach model DR 3900).

3.4.4. Biomass characterization

The algae biomass harvested at steady state of each operational condition was analyzed for the total nitrogen, protein, amino acid profile, total lipid, carbohydrate and ash content. Nitrogen content (%) was determined by Combustion Method (AOAC 990.03) using a Vario MAX Carbon Nitrogen analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Protein was calculated as nitrogen (%) $\times 6.25$. Ash was determined gravimetrically after heating at 550˚C for 16h. The amino acid profile was determined by Agricultural Experiment Station Chemical Laboratories, University of Missouri, based on AOAC Official method 982.30 E (a, b, c) Ch. 45.3.05.

3.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to test whether the cell growth condition significantly changes the cell density, cell productivity, and ammonia removal performance parameters. A statistical tool SAS (version 9.3, SAS Institute Inc., Cary, NC) was employed

with the *p*-value of 0.05 to determine the significant differences in results. The sample replicate was obtained by taking samples of certain growth condition for several days when the cell reached to the steady-state.

Chapter 4. Results and discussion

4.1. Algae cell growth

Microalgae can be found in a wide range of environment but each species has different environment preference and the growth rate can be maximized by finding the optimal growth condition. In this study, the cell growth at different conditions was first studied as an evaluation of algal ammonia removal capacity. Figure 4.1 shows the cell density of the entire continuous algal culture process performed in this project.

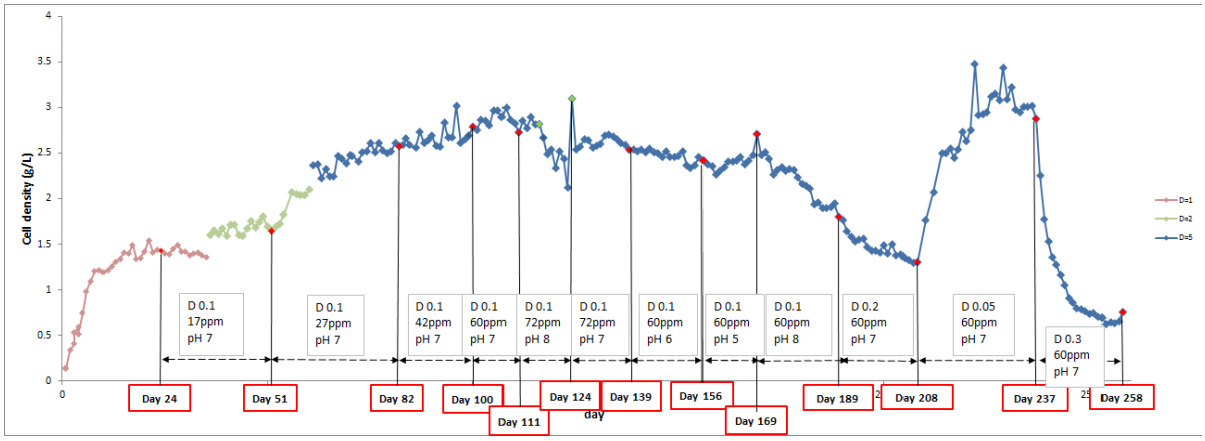

Figure 4. 1 Cell density of *S. dimorphus* during the continuous culture. The culture conditions in terms of the dilution rate (D, unit day^{-1}), ammonia concentration in the inlet gas and medium pH are indicated at different culture time points

4.1.1. Cell growth at different dilution rates

Dilution rate is an important factor for controlling the continuous culture performance. In general, the higher the dilution rate, the higher biomass productivity can be achieved. However, the highest dilution rate cannot exceed the maximal cell specific growth rate in order to avoid the cell-wash out. Therefore, in this project, we first performed a batch culture

with nitrogen as a limiting factor to determine the maximal cell specific growth rate, and thus, the highest dilution rate the continuous culture can be used.

The maximum specific growth rate was tested in 50mL Erlenmeyer flasks to determine the dilution rate range. By changing the initial NH_4-N concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.2 mM), the algal growth curve was obtained. The growth curve of each condition was converted to the logX (cell density) – N (nitrogen concentration) graph then the slopes of each concentration was used for the Monod equation. The growth dynamics expressed as Monod equation was:

$$\mu = \frac{\mu_{max} N}{k_s + N}, \mu_{max} = 0.35 \pm 0.01, K_s = 0.063 \pm 0.01 \quad \cdots\cdots\cdots\cdots \text{ Equation 4.1.}$$

where μ is the specific cell growth rate (day^{-1}), K_s is the half-saturation constant (mM), and N is nitrogen concentration (mM) (Figure 4.2). Therefore, the reactor was run at dilution rates of 0.05, 0.1, 0.2, and 0.3 day^{-1} to determine the optimal dilution rate.

Based on the result in Figure 4.2, the maximal specific growth rate of the cells was 0.35 day^{-1}, therefore, in the following continuous culture, the dilution rate was controlled at the range of 0.05 - 0.3 day^{-1}. Figure 4.3 shows dilution rate effect on both of the cell density and the cell productivity. The cell productivity was calculated as

$$\text{Biomass productivity} = \text{cell density} \times D \quad \cdots\cdots\cdots\cdots \text{ Equation 4.2.}$$

where D is the dilution rate (day^{-1}). The dilution rate effect was observed under inlet ammonia concentration being 60 ppm and medium pH being 7.0.

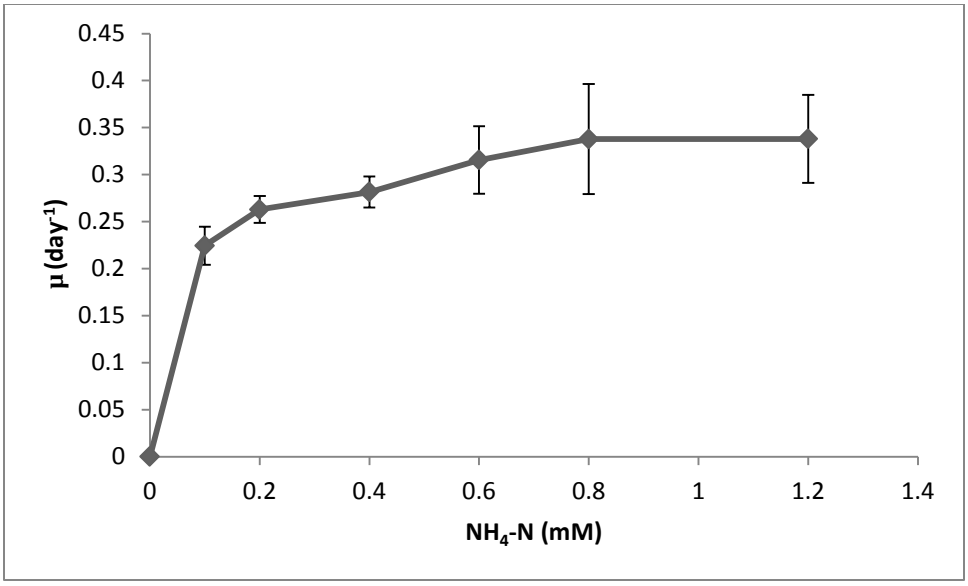

Figure 4. 2 Effect of initial ammonia-nitrogen concentration on the specific growth rate of *S. dimorphus*

As shown in the Figure 4.3, the cell density was highest at both of dilution rates of 0.05 day^{-1} (2.98 ± 0.05 g/L) and 0.1 day^{-1} (2.92 ± 0.07 g/L) and decreased with dilution rate increasing from 0.1 day^{-1} to 0.3 day^{-1}. The cell productivity also increased with the dilution rate decrease and was highest at a dilution rate of 0.1 (0.29 ± 0.007 g/L day) but diminished significantly (0.15 ± 0.003 g/L day) at 0.05 day^{-1}. Both of the cell density and productivity at different dilution rates were statistically different ($p < 0.0001$). The decreasing trend of cell density with dilution rate might be explained by the progression of cell washout. At each steady state, algae cell density maintained at a constant level as the cell growth rate keeps a pace with the dilution by a fresh medium. At higher dilution rate, however, more frequent exchange of fresh medium will make cells getting hard to keep their numbers in a unit volume.

The low cell productivity at 0.05 day^{-1} might be explained by the fact that the amount of nutrients remained in the reactor is not sufficient to support algal growth at low dilution rate. That is, other nutrients than nitrogen become a limiting factor for the algal growth so the cell production per unit time becomes slow down. Therefore, dilution rate of 0.1 day^{-1} is the

optimal condition for algae biomass production and this dilution rate was used for the following study on ammonia concentration and medium pH effects.

The different cell density throughout the varied dilution rates shown for *S. dimorphus* also observed for the same genus, *S. quadricauda.* In unialgal culture experiment of Takeya et al. (2004), *Microcystis novacekii* showed consistent cell number under different dilution rates while *Scenedesmus quadricauda* reach to different cell numbers throughout the changing of dilution rates [27].

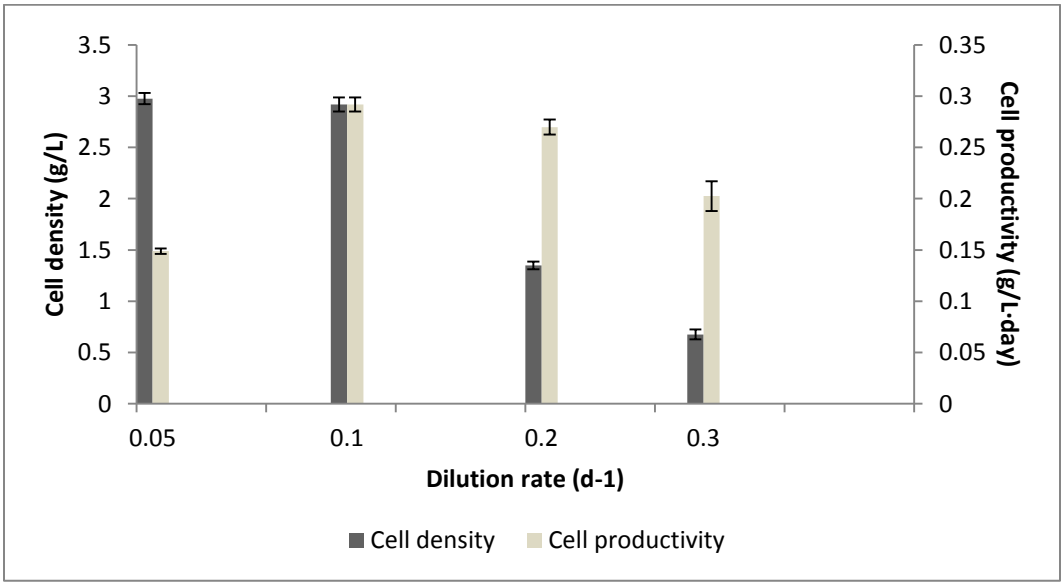

Figure 4. 3 Cell density and productivity of *S. dimorphus* under different dilution rates

4.1.2. Effects of ammonia concentration in the inlet gas on cell growth

Figure 4.4 presents the effect of ammonia concentration in the inlet gas on the *S. dimorphus* cell density and the cell productivity in continuous operation, with dilution rate and pH being controlled at 0.1 day^{-1} and pH 7, respectively. While all the terms in the ANOVAs were significant for all variables ($p < 0.0001$), the cell density and productivity increased with ammonia gas concentration increasing from 17 to 27 ppm and leveled off when ammonia gas

concentration increased from 27 to 72 ppm. The cell density and the cell productivity showed the same trend because the dilution rate was controlled to the same level. The highest cell density (2.92 ± 0.07 g/L) and productivity (0.29 ± 0.007 g/L day) were achieved at 60 ppm of ammonia concentration; while 17 ppm of ammonia concentration resulted in the lowest cell density (1.46 ± 0.03 g/L) and productivity (0.15 ± 0.003 g/L day). The lowest cell density and cell productivity at 17 ppm means the reactor was a nutrient-limiting environment.

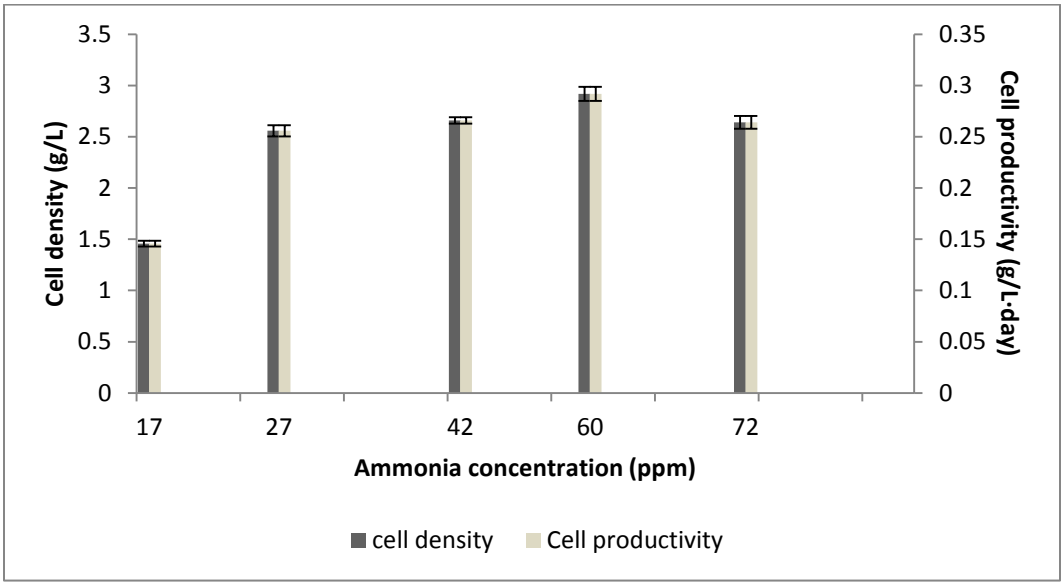

Figure 4. 4 Effects of ammonia concentration in the inlet gas on the cell density and productivity

To date, the effect of ammonia gas concentration on algal growth have not been well studied, but the utilization of ammonia from liquid phase by microalgae have been widely studied. The similar cell density and cell productivity obtained from 27 to 72 ppm ammonia gas is similar to the result of Tam and Wong (1996). In their batch scale study under neutral pH, *Chlorella vulgaris* showed similar cell number ($45.4 \pm 6.7 \times 10^6$ cells/mL) at the stationary phase for a wide range of ammonium concentration (20 mg/L to 250 mg/L) [31]. Similar explanation can be found in the *Chlamydomonas sp*. According to *Chlamydomonas* sourcebook, this algae can avoid any toxic effect of excessive intracellular amounts of

ammonium because it does not accumulate ammonium within intracellular compartments and cells excrete ammonium when their assimilation capacity is exceeded [32]. This inference will be justified by the consistent cell nitrogen absorption percentages covered in the section 4.3.2. However, in the study of Tam and Wong (1996), the cell number diminished when ammonium concentration reached to 1000 mg/L (29.18×10^6 cells/mL), indicating ammonia will result in an inhibition when its concentration exceeds a certain threshold level.

The upper limit of ammonia gas concentration and the pH effect should be carefully monitored because the un-ionized ammonia at higher concentrations is known to inhibit the cell growth of a wide range of algae species [22]. The upper level of non-inhibitory ammonia concentration has been intensively studied. Abeliovich and Azov (1976) observed growth inhibition of *Scenedesmus obliquus* above 2.0 mM (28ppm) of NH_3 concentration at pH values over 8.0 [22]. Park et al. (2010) observed *S. accuminatus* growth at 100, 200, 400, 500, 800, 1000 ppm of NH_4-N at pH 8.4 and concluded that the algae cell density was highest at 100ppm, but didn't consider below 100ppm [24].

It is also notable that from 72ppm, the pH drifted up so the pH had to be adjusted repeatedly (Figure 4.5). This result is different from the report by Tam and Wong (1996), in which the pH of the culture solution decreased below to 4 when *C. vulgaris* was provided with ionized ammonium more than 50 ppm [31]. This dissimilarity can be explained by Equations 4.3, 4.4 and 4.5.

$$NH_4^+ (l) \leftrightarrow NH_3(l) + H^+ \quad \cdots\cdots\cdots\cdots \text{ Equation 4.3.}$$

$$\mathrm{NH_3(g) \leftrightarrow NH_3\ (l)} \quad \cdots\cdots\cdots\cdots \text{ Equation 4.4.}$$

$$\mathrm{N}H_3\ (aq) + \ H_2\mathrm{O(l)} \leftrightarrow \mathrm{N}H_4^+(aq) + \mathrm{O}H^-(aq) \quad \cdots\cdots\cdots\cdots \text{ Equation 4.5.}$$

Equation 4.3 is account for the diminished pH of Tam and Wong (1996). The excess amount of ammonium (NH_4^+) that is not assimilated by algae will dissociate into NH_3 (l) and H^+ ion depending on the equilibrium constant, resulting in the pH decrease. On the other hand, the raised pH of this experiment can be explained by Equations 4.4 and 4.5. According to the

Equation 4.4, the ammonia gas (NH_3) fed into the medium will first make equilibrium with NH_3 (l) and will be consumed by algae. When ammonia is added above the amount that algae can assimilate, the ammonia residues will go through Equation 4.5, which generates hydroxide ion (OH^-), making the higher medium pH.

As shown in section 4.1.3., the algae cell growth is retarded at above neutral pH. Therefore, ammonia concentration in the inlet gas of 72 ppm is not favorable to algal-ammonia scrubber system because of using acid for pH controlling, rather than the ammonia growth inhibitory effect.

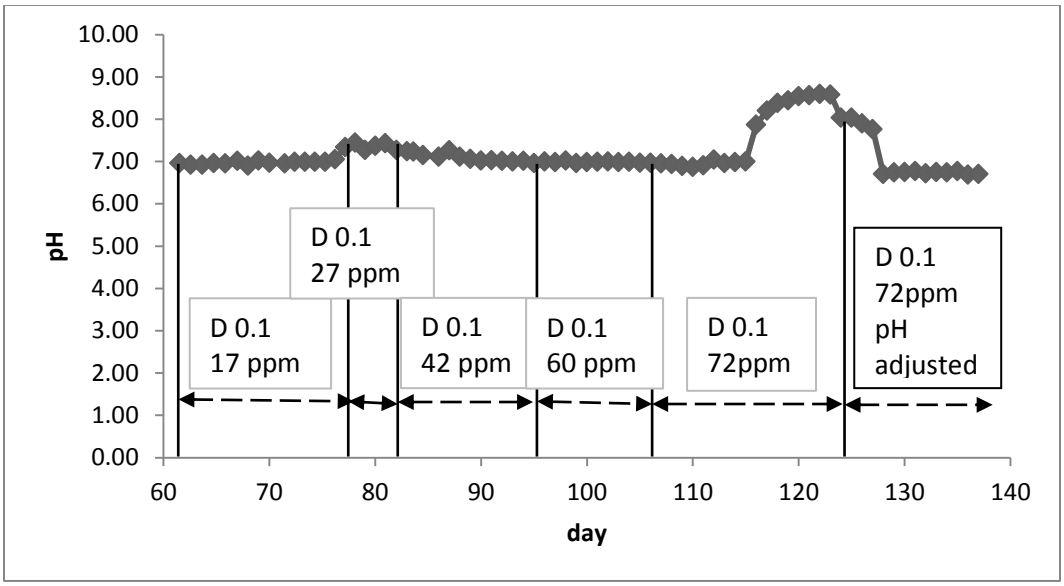

Figure 4. 5 Medium pH level in continuous algal culture when sparging ammonia-laden air at different ammonia concentrations

4.1.3. Effect of medium pH on algal growth

Medium pH is an important parameter to affect algal growth because it determines both of ammonia gas dissolution and algal metabolic activities. The effect of pH was examined under dilution rate of 0.1 day^{-1} with ammonia concentration of 60 ppm. The cell density and productivity from different pHs were statistically different ($p < 0.0001$). As shown in Figure

4.6, the highest cell productivity was shown at pH 7 (0.29 ±0.007 g/L day), following pH 5 (0.24 ±0.004 g/L day), 6 (0.24 ±0.007 g/L day), and lowest at 8 (0.19 ±0.003 g/L day). The cell productivity at pH 5 and 6 were similar but significantly low at pH 8. This result of pH effects may be explained by Azov and Goldman (1982) that the ammonia inhibition is related to the dissociation of NH_4^+ as a function of pH, because the ionized ammonia cannot penetrate freely through the algal cell membrane. The penetrated ammonia will elevate the cell's internal pH to an inhibitory level [22, 23]. Figure 4.6 also implies that *S. dimorphus* grows best at the neutral pH and overdose of acid will not be helpful for improving the cell growth performance although the ammonia adsorption at the lower pH will increase.

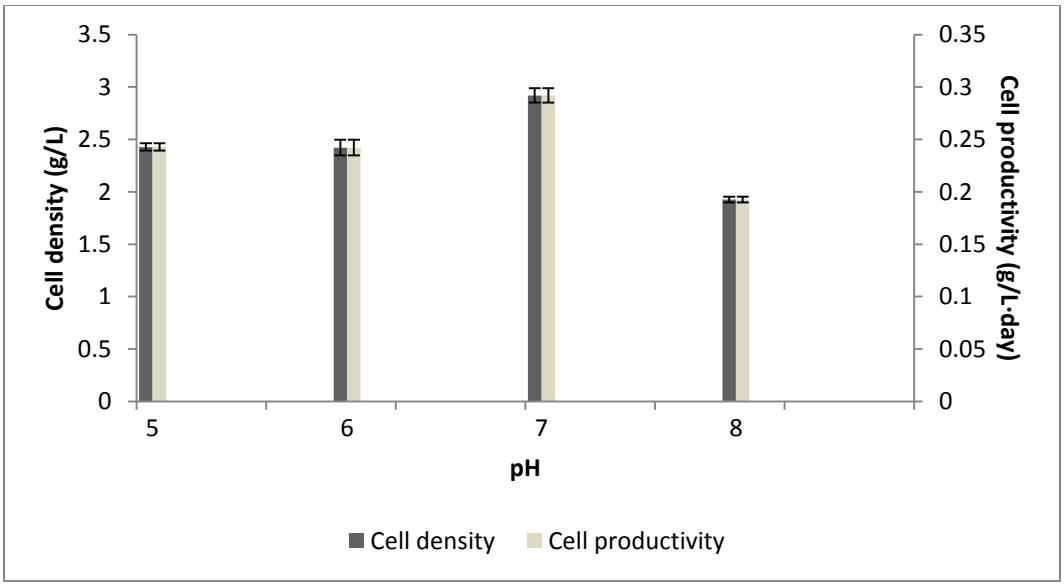

Figure 4. 6 Effects of medium pH on cell density and productivity

4.2. Ammonia gas removal performance

The ammonia gas removal performance was evaluated through criteria of i) volumetric ammonia removal capacity, ii) cellular ammonia consumption rate, iii) cell yield, iv) ammonia gas removal percentage (%), and v) N conversion efficiency. The concept and calculation procedures for those four parameters are shown as follows:

i) Volumetric ammonia removal capacity (ΔNH_3/L (g/L day))

The volumetric ammonia removal capacity implies how much ammonia gas can be removed per unit algae suspension volume in a day and can be applied to the design of a reactor. It was calculated by the following equation.

$$\frac{NH3_{in} - NH3_{out}\ (g/day)}{Total\ reactor\ volume\ (L)} \quad \cdots\cdots\cdots\cdots \text{ Equation 4.6}$$

ii) Cellular ammonia consumption rate ($\Delta NH_3/\Delta x \cdot L$ (g NH_3/g cell · day))

The cellular ammonia gas consumption rate indicates the individual algae cell capacity to consume ammonia gas in a day. It was calculated by the following equation.

$$\frac{volumetric\ ammonia\ removal\ capacity\ (g/L \cdot day)}{cell\ density\ (g/L)} \quad \cdots\cdots\cdots\cdots \text{ Equation 4.7}$$

iii) Cell yield ($\Delta x / \Delta NH_3$ (g cell/g NH_3))

The cell yield was determined by following equation, implying how many cell biomasses can be produced by unit ammonia gas. i.e.,

$$\frac{cell\ productivity\ (g/L \cdot day)}{volumetric\ ammonia\ removal\ capacity\ (g/L \cdot day)} \quad \cdots\cdots\cdots\cdots \text{ Equation 4.8}$$

Contrary to the cellular ammonia consumption rate, the cell yield used cell productivity instead of cell density because the cellular ammonia consumption rate focuses on the ability of individual cells while the cell yield focuses on the amount of the entire cell production.

iv) Ammonia gas removal rate (%)

The ammonia gas mitigation efficiency of the algal-ammonia scrubber can be measured as follows.

$$\frac{NH3_{in} - NH3_{out}\ (g/day)}{NH3_{in}\ (g/day)} \times 100 \quad \cdots\cdots\cdots\cdots \text{ Equation 4.9}$$

4.2.1. Effect of dilution rate

4.2.1.1. Volumetric ammonia removal capacity (ΔNH_3/L (g/L·day))

As shown in Figure 4.7, the volumetric ammonia removal capacity was not affected (0.04 ± 0.002 g/L·day) by dilution rate change because the ammonia gas inlet and the reactor volume were constant while the ammonia gas outlet difference between different dilution rates was negligible (d = 0.002). This implies that the algal capacity of consuming ammonia gas per unit volume is not influenced by the frequency of fresh medium exchange. However, the volumetric ammonia removal capacity difference between dilution rates was statistically significant ($p < 0.0001$) because of too narrow variance in each dilution rate.

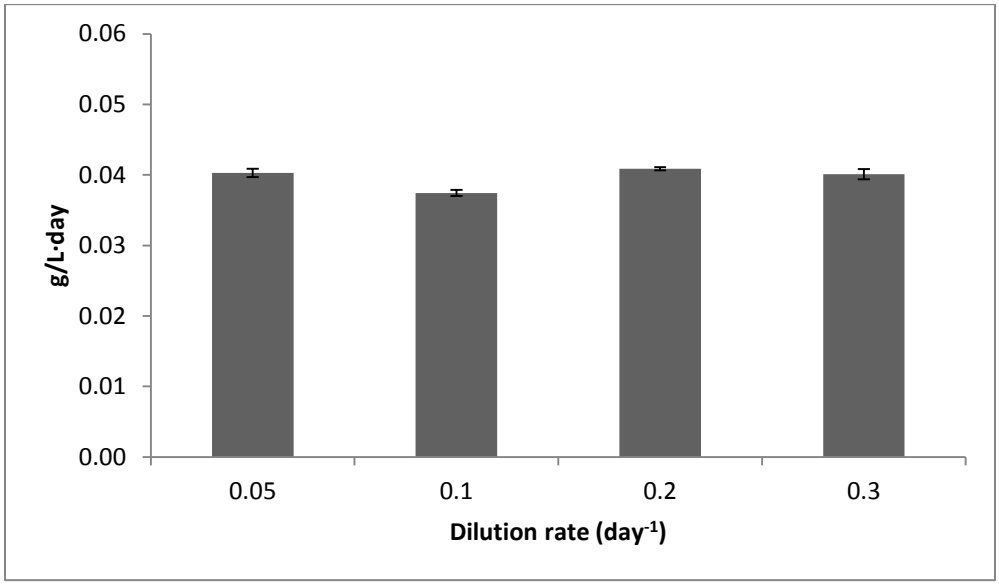

Figure 4. 7 Dilution rate effect on the volumetric ammonia removal capacity

4.2.1.2. Cellular ammonia consumption rate ($\Delta NH_3/\Delta x \cdot L$ (g NH_3/g cell·day))

Figure 4.8 shows that the cellular ammonia consumption significantly changed with the dilution rate shift from 0.1 day^{-1} to 0.3 day^{-1}. Because the cellular ammonia consumption rate is derived from volumetric ammonia removal capacity divided by cell density and the

volumetric ammonia removal capacity was unchanging throughout the dilution rates, the cellular ammonia consumption rate is directly proportional to the inverse of the cell density. It is also notable that significantly high cellular ammonia consumption rate throughout the whole growth condition was shown at dilution rate 0.3 day^{-1} (0.06 $\pm$ 0.01 g NH_3/g cell·day) and 0.2 day^{-1} (0.03 $\pm$ 0.00 g NH_3/g cell·day), at which the lowest cell densities appeared. Similar result can be found in Tam and Wong (1996), in which *Chlorella vulgaris* was used to remove a wide range of ammonium concentrations (0-1000 mg/L) at neutral pH. In their study, the highest ammonium uptake rate was recorded in the medium containing 1000 mg-N/L, at which the cell number started to diminish [31].

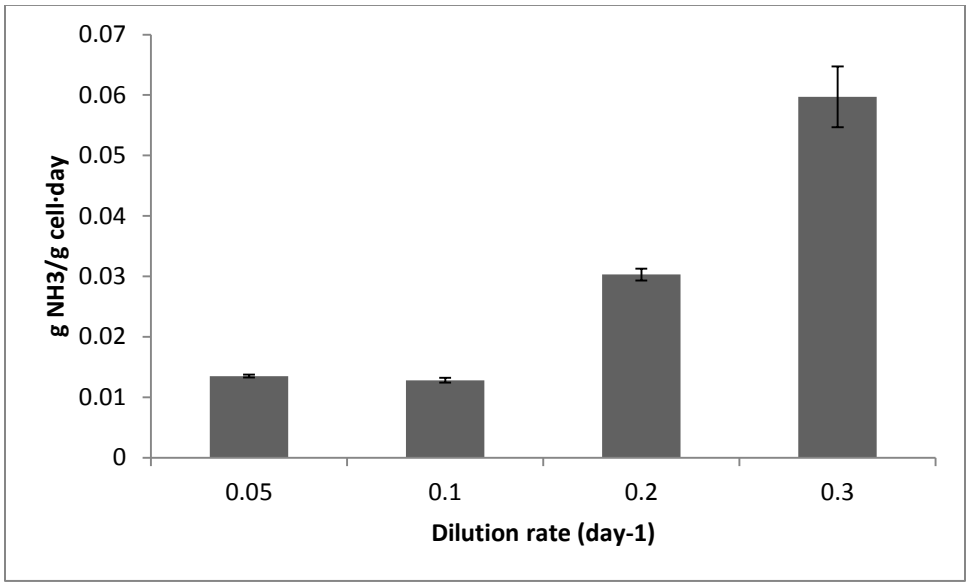

Figure 4. 8 Dilution rate effect on the cellular ammonia consumption rate

4.2.1.3. Cell yield (Δx / ΔNH_3 (g cell/g NH_3))

As shown in Figures 4.9, 4.13, and 4.17, 0.1 day^{-1} produced the highest cell yield among the four dilution rates (7.80 $\pm$ 0.24 g cell/g NH_3) and 0.05 day^{-1} (3.69 $\pm$ 0.06 g cell/g NH_3) showed the lowest cell yield throughout the whole experiment. The cell yield was greatly affected by the cell productivity because of the constant volumetric ammonia removal capacity throughout the dilution rate experiments.

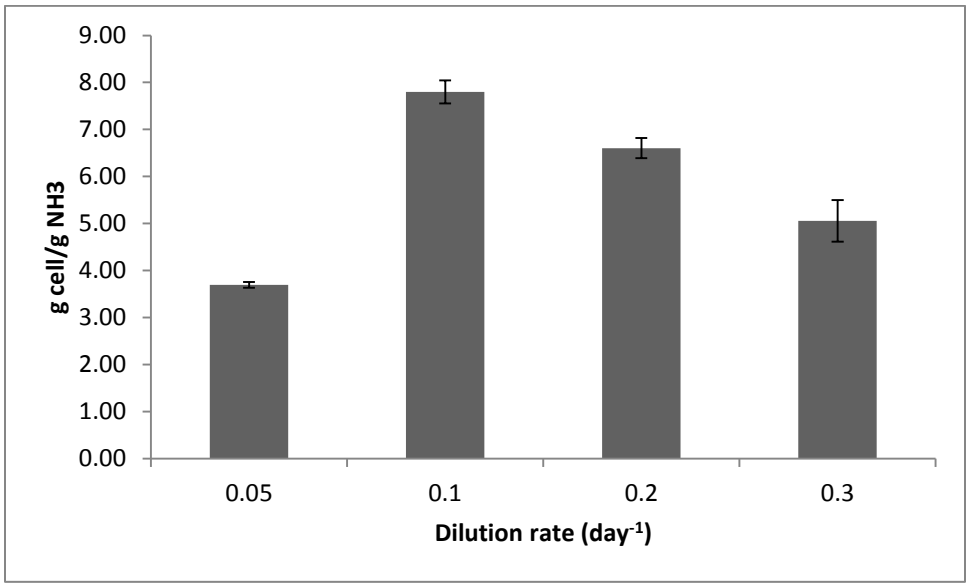

Figure 4. 9 Dilution rate effect on the cell yield

4.2.1.4. Ammonia gas removal ratio (%)

As shown in Figure 4.10, the ammonia gas removal rate was always high above 93% up to 96 % throughout all the dilution rates, therefore was not quite affected by the dilution rate. However, the ammonia gas removal ratio difference between dilution rates was statistically significant ($p < 0.0001$) because of too narrow variance in each dilution rate.

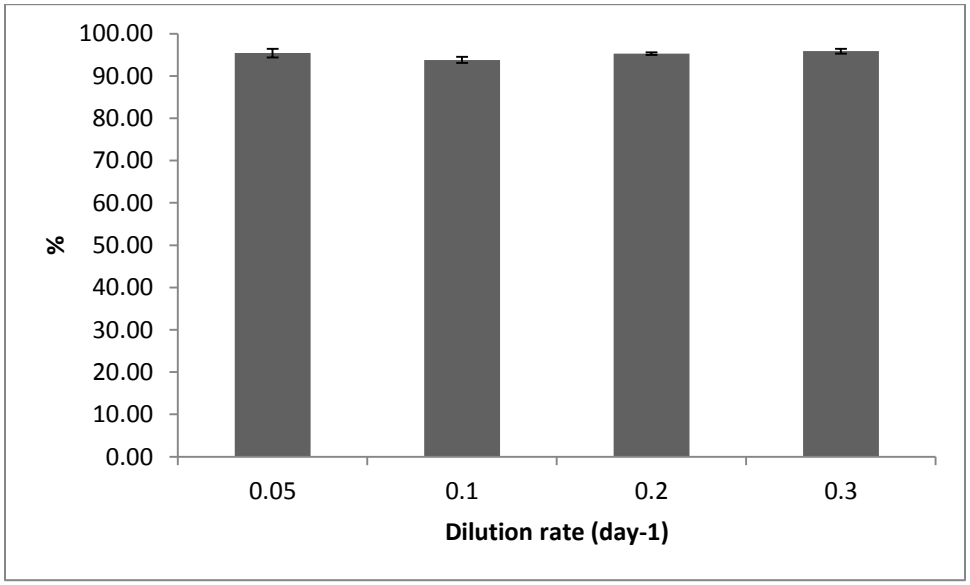

Figure 4. 10 Dilution rate effect on the ammonia gas removal rate

4.2.2. Effect of ammonia concentration

4.2.2.1. Volumetric ammonia removal capacity (ΔNH_3/L (g/L·day))

As shown in Figure 4.11, the ammonia removal per unit volume improved with the increase of the ammonia gas concentration (correlation coefficient, R= 0.99). It was improved about 6.6 times from 17 ppm to 72 ppm (0.0077 g/L·day at 17 ppm; 0.051 g/L·day at 72 ppm). This trend is caused by the stable ammonia gas outlet concentration and constant algae biomass volume throughout the inlet ammonia concentration experiment.

The ascending ammonia removal capacity with increasing inlet concentration is also found in the biofilter. H. Jorio et al. (2000) showed that under constant gas flow rate, the elimination capacity increases as the inlet concentration is increased until the optimal inlet concentration reached. [33]. N.J. Kim et al. (2000) also showed linear relationship between ammonia load of 42-290 ppm_v and removal capacity in the biofilter [20].

The actual volumetric ammonia removal capacity value is also comparable to the biofilter. Y. Liang et al. (2000) calculated volumetric ammonia removal capacity of the biofilter under ammonia concentrations of 20-500 ppm_v: 0.0195-0.0203 g/L·day at 20 ppm_v and 0.0758-0.0764 g/L·day at 100 ppm_v [34].

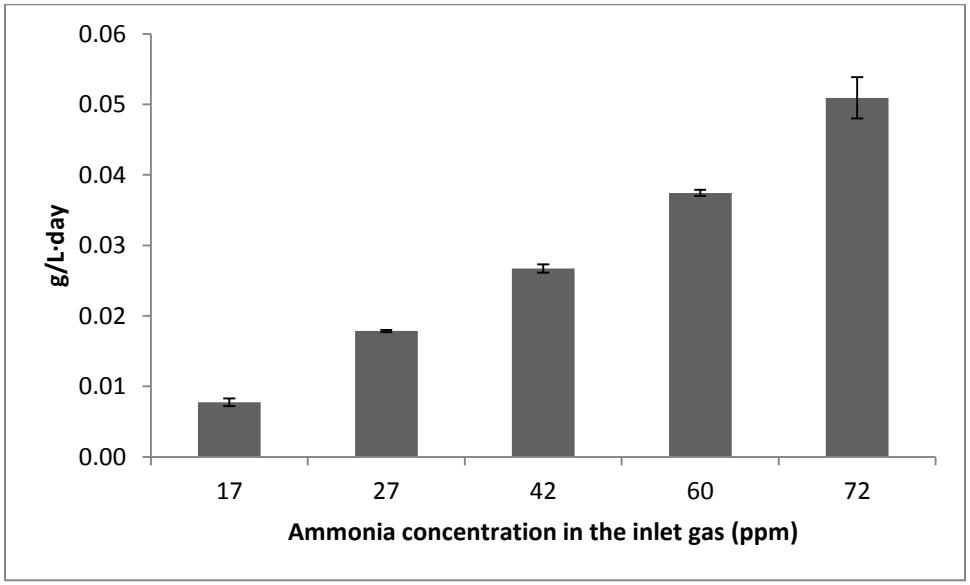

Figure 4. 11 Effect of ammonia concentration on the volumetric ammonia removal capacity

4.2.2.2. Cellular ammonia consumption rate ($\Delta NH_3/\Delta x \cdot L$ (g NH_3/g cell·day))

As shown in Figure 4.12, the cellular ammonia consumption rate was substantially affected by the ammonia concentration in the inlet gas (correlation coefficient, R = 0.97). This is because of the dependency of volumetric ammonia removal capacity on the ammonia concentration in the inlet gas and relatively stable cell density throughout the ammonia concentration change. The proportional increase of the cellular ammonia consumption rate along with the increasing ammonia concentration of present study is in consistent with the ammonium concentration effect reported by Tam and Wong (1996). They stated that the specific ammonium uptake (mg N uptake per cells) increased with initial ammonium concentrations [31].

The increasing cellular ammonia consumption rate with the increasing ammonia concentration in the inlet gas implies that the algae cell can utilize nitrogen as much as possible and 72 ppm of the ammonia gas concentration in the inlet gas is not a growth inhibitory level.

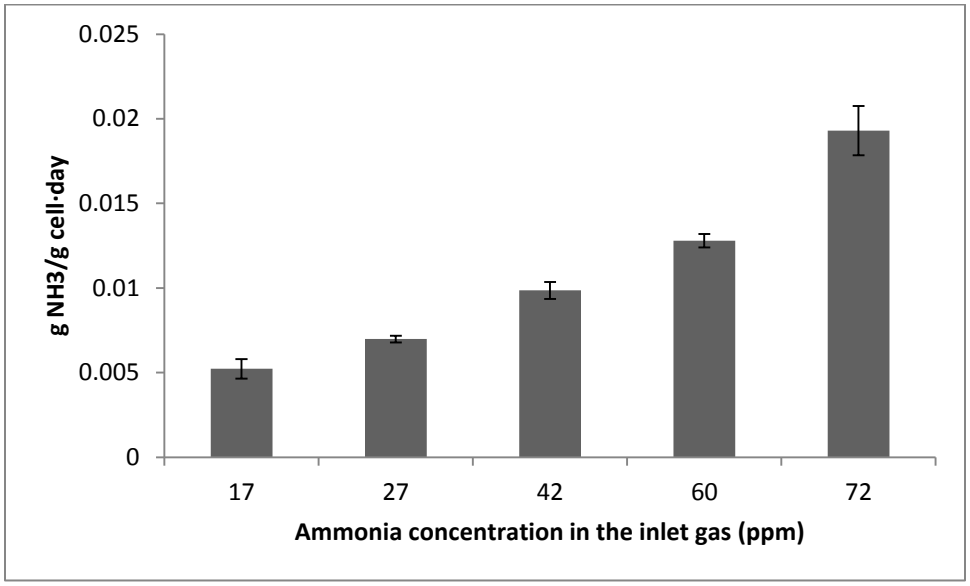

Figure 4. 12 Effect of ammonia concentration on the cellular ammonia consumption rate

4.2.2.3. Cell yield (Δx / ΔNH_3 (g cell/g NH_3))

The cell yield also correlated with the ammonia concentration in the inlet gas (correlation coefficient R= - 0.97). It is notable that the highest cell yield throughout the whole experiment was obtained at 17 ppm of ammonia concentration (19.4 $\pm$ 2.52 g cell/g NH_3), because the cell productivity was not that low relative to the volumetric ammonia removal capacity than other cases.

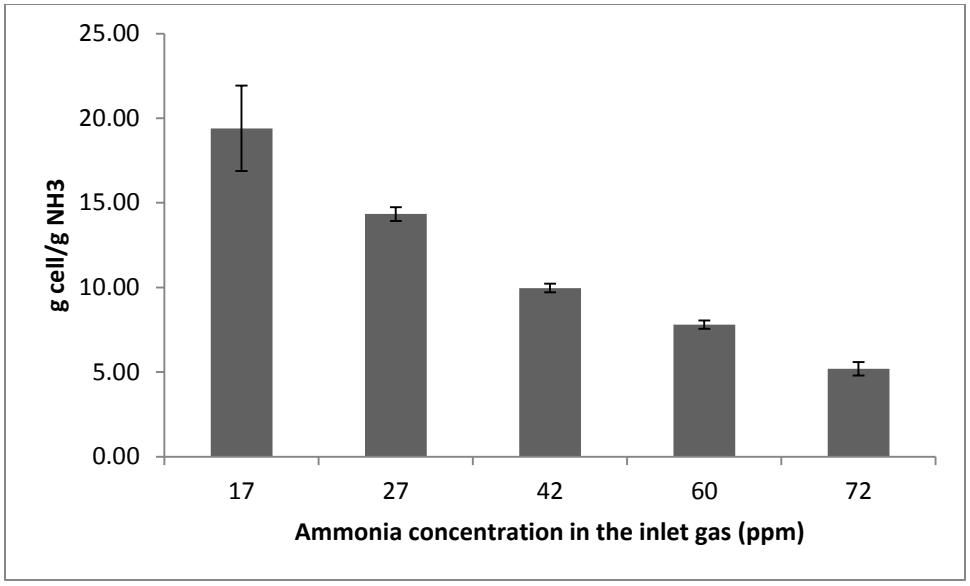

Figure 4. 13 Effect of ammonia concentration on the cell yield

4.2.2.4. Ammonia gas removal ratio (%)

The ammonia gas removal ratio was constantly high above 90% for all conditions except 17 ppm (84.5 %). The volumetric ammonia removal capacity difference between dilution rates was statistically significant ($p < 0.0001$) because of too narrow variance in each dilution rate. Similar result can be found in other literatures. L. E. Gonzalez et al. (1997) used *Scenedesmus dimorphus* to treat 36.3 mg/L of ammonium in an aerated bioreactor with 2-L working volume and obtained 95% of ammonium removal efficiency [25]. Tam and Wong (1996) also removed more than 95% of ammonium using *Chlorella vulgaris* in flasks for initial ammonium concentrations of 40-80 mg/L. In contrast to present study, however, 100% of ammonium was absorbed by algae for 10-20 mg/L initial ammonium [31].

The different ammonia or ammonium removal percentages at low ammonia or ammonium concentration might arise from the different experimental design: present study exchanged the medium continuously and the reactor had a ventilation hole whereas Tam and Wong (1996) inoculated algae in closed flasks that contain different initial ammonium concentrations. Therefore, the unabsorbed ammonia gas could escape from the reactor while

the unabsorbed ammonium stays in the medium and the algae eventually utilize as much of it as possible for their growth. The high percentage of ammonia outlet in the nitrogen mass balance confirms this inference (section 4.3.2.).

The ammonia gas removal ratio of other end-of-pipe ammonia mitigation methods, such as acid scrubber and biofilter, shows similar result. The ammonia removal performance of those systems is generally high up to 100 % depending on the inlet concentration, packing material, and operating conditions [35]. J. R. Kastner (2004) tested biofilter with low ammonia concentration (0-25 ppm_v) and the ammonia removal efficiency obtained was 70-100 % for ammonia inlet concentration of 8-25 ppm_v and 30-80 % for 0-6 ppm_v [36]. The lower ammonia removal efficiency at low ammonia inlet concentration concurs with our result.

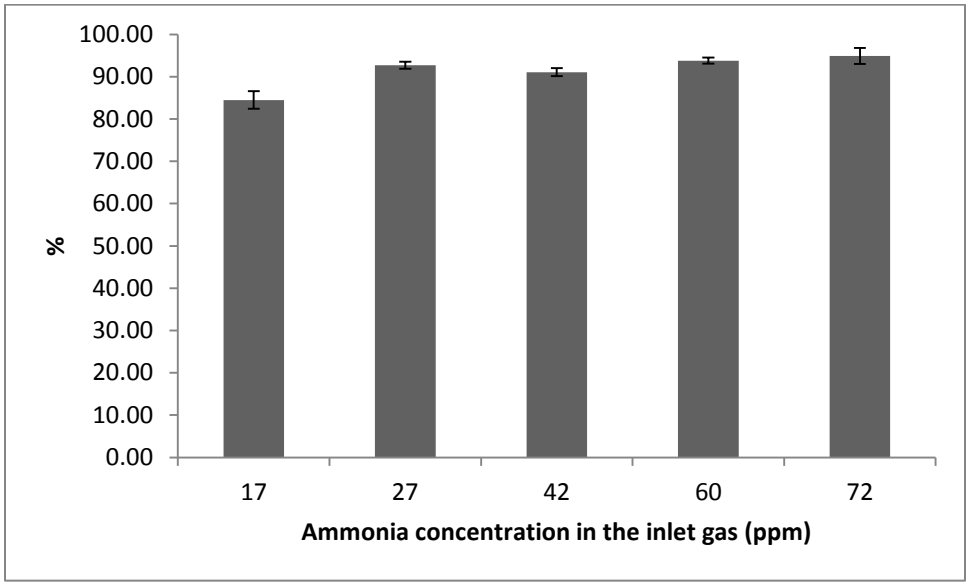

Figure 4. 14 Effect of ammonia concentration on the ammonia gas removal rate

4.2.3. Effect of medium pH

4.2.3.1. Volumetric ammonia removal capacity (ΔNH_3/L (g/L ·day))

Although the pH determines the dissolution of ammonia gas into the liquid medium and the differences were statistically significant ($p < 0.0001$) because of the too narrow variance in each dilution rate, the volumetric ammonia removal capacity turned out to be not affected (0.04 ± 0.002 g/L day) by pH change. This is primarily because the ammonia gas inlet and the reactor volume were controlled to the same level. The ammonia outlet at pH 8 was higher than other cases but the absolute amount of difference became negligible when it was divided by the reactor volume (5L).

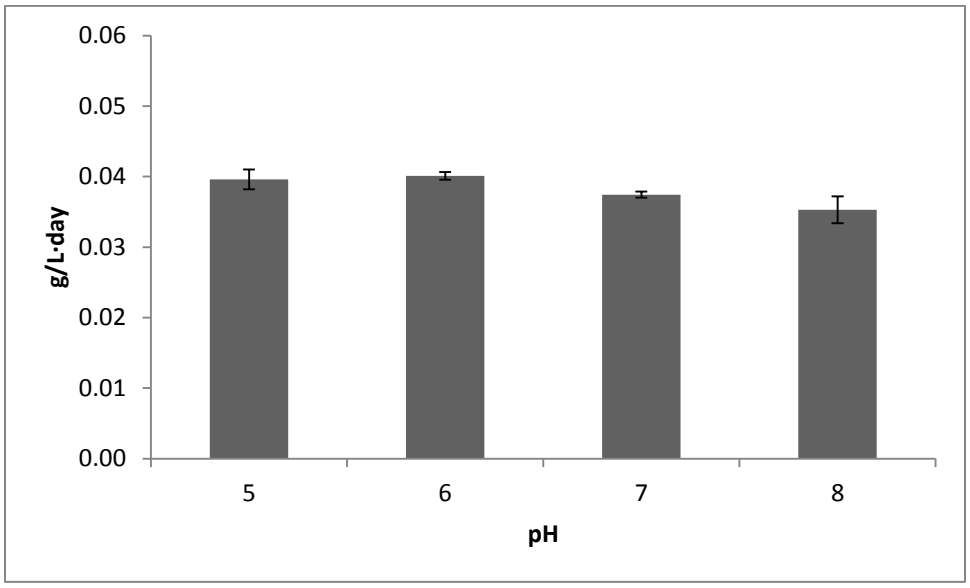

Figure 4. 15 Effect of medium pH on the volumetric ammonia gas removal capacity

4.2.3.2. Cellular ammonia consumption rate ($\Delta NH_3/\Delta x \cdot L$ (g NH_3/g cell day))

The cellular ammonia consumption rate did not show significant correlation with the pH change. The lowest value was observed at pH 7 because the cell density was highest at this condition under the stable volumetric ammonia removal capacities.

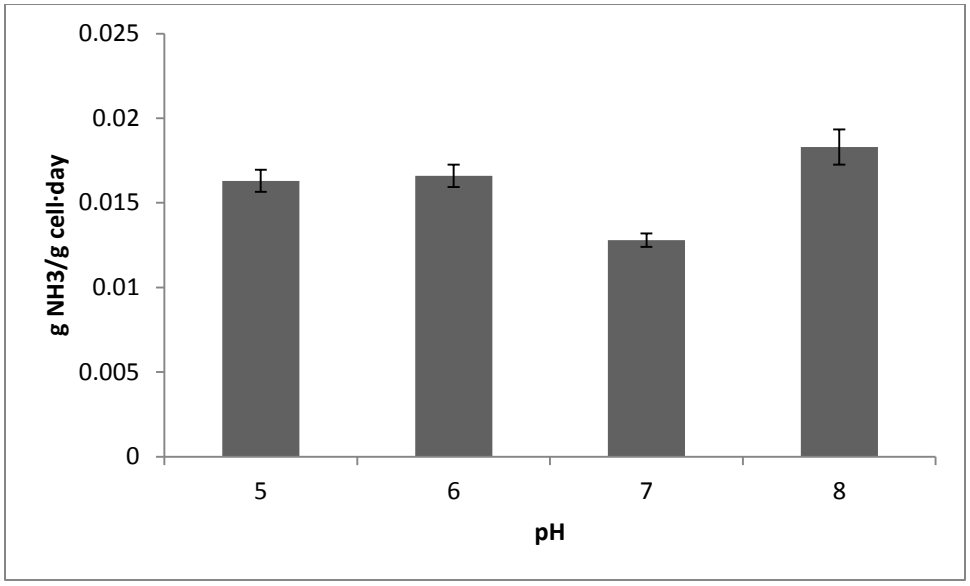

Figure 4. 16 Effect of medium pH on the cellular ammonia consumption rate

4.2.3.3. Cell yield ($\Delta x / \Delta NH_3$ (g cell/g NH_3))

The cell yield showed similar trend with the cell productivity because of the constant volumetric ammonia removal capacities throughout all pH cases. Therefore, the highest cell yield was observed at the optimal condition (pH 7, 7.80 $\pm$ 0.24 g cell/g NH_3).

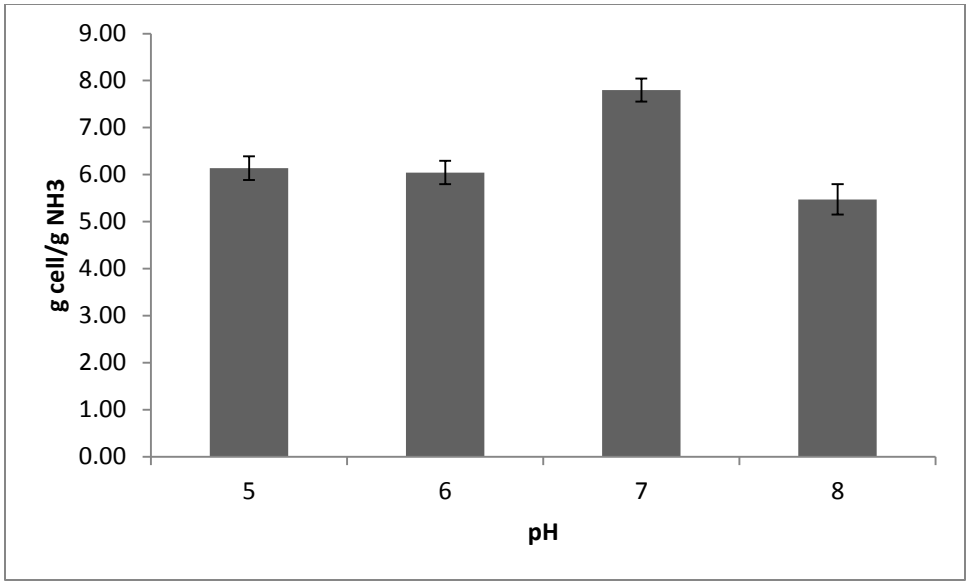

Figure 4. 17 Effect of medium pH on the cell yield

4.2.3.4. Ammonia gas removal ratio (%)

The ammonia gas removal ratio was constantly high above 93 % for all conditions except when the medium was basic (pH 8, 83.0 %). The low ammonia removal efficiency at pH 8 is because of the higher ammonia outlet ventilation than other cases.

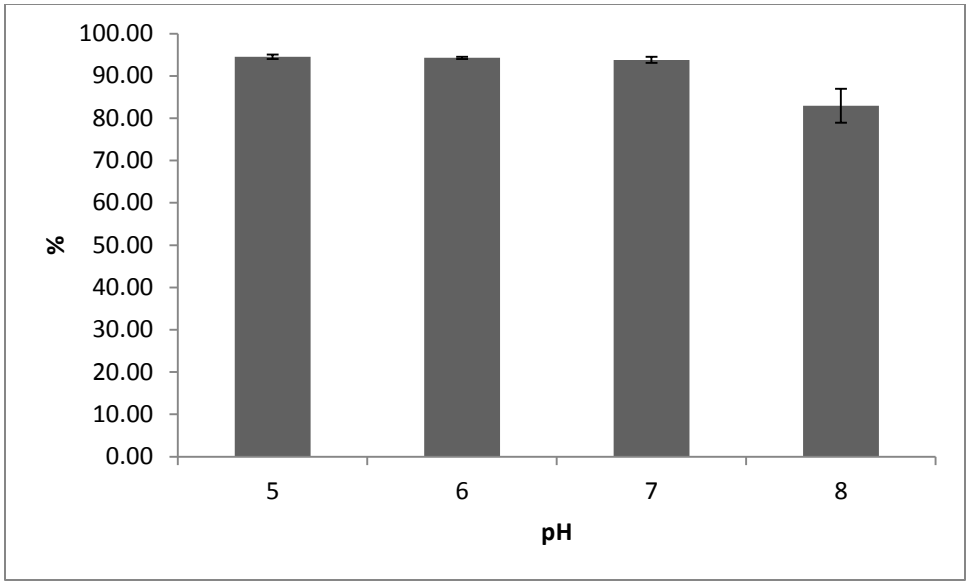

Figure 4. 18 Effect of medium pH on the ammonia gas removal rate

4.3. The fate of ammonia in inlet gas - nitrogen mass balance

In order to chase the nitrogen fate, nitrogen mass balance was calculated for each condition. The nitrogen mass balance equation used was (g/day):

$$N_{input} = N_{cell\ absorption} + N_{liquid\ dissolve} + N_{outlet} + \varepsilon \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \text{Equation 4.10}$$

Each term was calculated by those equations.

$$N_{input}\ (g/day) = NH_{3input}\ (L/min) \times NH_3\ density\ (g/L) \times 60 \times 24\ (min/day) \times 14/17 \quad \cdots\cdots \text{Equation 4.11}$$

$$N_{cell\ absorption}\ (g/day) = cell\ density\ (g/L) \times V(L) \times D\ (day^{-1}) \times N\ content\ (\%) \quad \cdots\cdots \text{Equation 4.12}$$

$$N_{liquid\ dissolve}\ (g/day) = (nitrate\ (g/L) + ammonia\ (g/L)) \times V\ (L) \times D\ (day^{-1}) \quad \cdots\cdots \text{Equation 4.13}$$

$$N_{outlet}\ (g/day) = Air_{input}\ (L/min) \times ammonia\ concentration\ (10^{-6}) \times 14/17 \quad \cdots\cdots\cdots \text{Equation 4.14}$$

where V is reactor volume and D is dilution rate.

4.3.1. Effect of dilution rate

Table 4.1 shows the nitrogen mass balance at different dilution rates. The highest cell absorption occurs at the optimal growth condition (D = 0.1 day^{-1}), following in order of 0.2, 0.3, and 0.05 day^{-1}. This tendency of cell N absorption can be understood in a similar way to the cell productivity covered in section 4.1.1.

Table 4. 1 Nitrogen mass balance at dilution rate

(%)	D 0.05	D 0.1	D 0.2	D 0.3
Input	100	100	100	100
Cell absorption	52.7	98.6	77.8	65.3
Liquid	37.7	0.064	10.0	20.7
Outlet	7.72	5.95	5.67	5.39
error	1.95	-4.66	6.53	8.54

The effects of dilution rate and pH were tested after ammonia concentration test (controlled at pH 7 and 0.1 day^{-1}) in order of pH 6, 5, and 8 (controlled at 60 ppm and 0.1 day^{-1}), and dilution rate of 0.2, 0.05, and 0.3 day^{-1}(controlled at 60 ppm and pH 7). After testing ammonia concentration of 72 ppm, which led to an over-nutrient medium state, there always remained significant amount of ammonia in the medium liquid. From this observation, it can be concluded that the high ammonia concentration has a long term effect on the liquid N absorption.

The liquid N absorption increased as the cell N absorption decreased: when the cell absorption was poor, the liquid became highly over-nutrient state, which can be a good habitat for other organisms. For this reason, adjusting dilution rate to the optimal condition is important not only because of high cell productivity, but also because of contamination prevention.

The ammonia outlet was consistent through all dilution rate cases. Thus, the ammonia gas ventilation is not affected by the frequency of medium exchange.

4.3.2. Effect of ammonia gas concentration

Table 4.2 summarizes the nitrogen mass balance at different ammonia gas concentrations. At the optimal growth condition (60 ppm), the portion of cell absorption is highest and the ammonia outlet that was not trapped by anywhere is lowest. Therefore, controlling the algae growth condition to the optimal condition is important for greater algal nitrogen assimilation and algal protein production.

Although the cell density and productivity of 17 ppm was prominently low, the cell N absorption portion was similar to the 72 ppm and lower than other cases (27, 42, and 60 ppm). The similar cell N absorption of 17 ppm and 72 ppm contrasts with the result of cellular ammonia consumption rate in section 4.2.2., which showed the highest cellular ammonia consumption rate at 72 ppm. This contrast is because the cellular ammonia consumption rate does not reflect the cellular ability to consume the ammonia gas; rather, it focuses on the ammonia removal amount. In other words, if the cell could maintain their cell density and productivity nevertheless of the depreciation of the cellular ammonia consumption ability at 72 ppm, the cellular ammonia consumption rate could be increased with the increased ammonia concentration. The cell density and productivity maintenance at 72 ppm implies that the algae adapt to their environment by changing their biochemical composition in the cell. This also implies that the nitrogen mass balance is more accurate parameter to know the cellular ammonia uptake ability.

It is also interesting to compare the liquid and outlet of 17 ppm and 72 ppm; the outlet portion is significantly high at 17 ppm, while most of the unabsorbed ammonia gas goes to the liquid portion rather than the outlet portion at 72 ppm. This is primarily because of the constantly low ammonia gas ventilation amount throughout the all ammonia concentrations and also because the medium capacity is enough to capture the ammonia gas so the liquid

ammonia-ammonium equilibrium (Equation 4.5) occurs quickly. The medium liquid capacity of ammonia capture was ignorable (below 0.2 %) up to 60 ppm, but was escalated to 20.3 % at 72 ppm, resulting in an over-nutrient state.

Although the nitrogen mass balance was carefully and meticulously calculated, the error term for the low ammonia conditions were prominent. Ammonia that could not be accounted for might be attributed to: (a) experimental measurement error; (b) possible ammonia gas leaking from the reactor; and (c) nitrogen assimilation by other microorganisms such as rotifer or bacteria.

Table 4. 2 Nitrogen mass balance at different ammonia gas concentration in inlet gas

(%)	17 ppm	27 ppm	42 ppm	60 ppm	72 ppm
Input	100	100	100	100	100
Cell absorption	77.4	90.9	82.5	98.6	72.7
Liquid	0.172	0.0941	0.0564	0.0637	20.3
Outlet	20.4	8.06	10.9	5.95	5.43
error	2.05	0.926	6.55	-4.66	1.56

4.3.3. Effect of medium pH

Table 4.3 represents the pH effect on the nitrogen distribution. Besides the optimal pH, the cell N absorption was markedly low and the liquid N absorption was high. The low cellular N absorption at pH 5, 6, and 8 reflects the retarded cellular N absorption ability and also implies that the cellular metabolism of the algae is optimum at the neutral pH. The high liquid N absorption at pH 5 and 6 reflects the most of ammonia exists as ammonium following the equilibrium of Equation 4.5.

The unutilized ammonia by cell was mostly evacuated at pH 8. This also can be explained by the pH effect on the ammonia-ammonium equilibrium. As shown in Figure 2.4 in Chapter 2,

the majority of ammonia exists as ammonium below pH 7; ammonia is getting increase while ammonium is getting decrease after pH 7; and ammonia and ammonium are the same amount at pH 9.4. Likewise, the ammonia outlet was indistinct under pH 7 whereas became significant at pH 8 in present study.

Table 4. 3 Nitrogen mass balance at different medium pH

(%)	pH 5	pH 6	pH 7	pH 8
Input	100	100	100	100
Cell absorption	64.4	68.1	98.6	55.6
Liquid	31.6	36.1	0.0637	16.6
Outlet	6.52	6.52	5.95	26.4
error	-2.55	-10.8	-4.66	1.43

The data in tables 4.1, 4.2, and 4.3 show that the majority of nitrogen was absorbed by the cell. This phenomenon concurs with nitrogen mass balance study conducted by O. R. Zimmo et al. (2004) using ammonium in algae pond at different seasonal periods, concluded that the largest nitrogen flux was algae biomass sedimentation [37]. This implies that, in order to mitigate more ammonia gas the higher algae cell growth is important.

It is also noticeable that the trend of cell absorption is in consistent with the tendency of cell productivity (g/L ⋅day) rather than the cell density (g/L) in all cases. This means that the cell absorption capacity depends on the duration time that the algae exposed to the ammonia.

4.4. Algae biomass characterization: amino acid composition

The protein is composed of different amino acids hence the high quality of dietary protein can be assured by the balance of amino acids between the absorbed one and the required one by animal. Because the surplus nitrogen of essential amino acids (EAAs) remains in the body and can be used in the synthesis of non-EAAs, an ideal protein is the one that provides the

exact balance of amino acids needed for optimum performance and maximum growth [38]. Feeding the ideal protein is beneficial for abating ammonia gas because it minimizes nitrogen excretion from animal.

The actual amino acid constitution of present study was compared with *S. obliquus* from published data in table 4.4. The growth condition of Becker (1984) was urea 60 ppm and NH_4^+ 20 ppm, controlled pH to the unknown level in open pond [39]; Osman (2004) was KNO_3 140 ppm, pH 6, periodic dilution [40].

The essential amino acids (EAAs) arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, methionine, and valine and non-EAA tyrosine, cysteine, aspartic acid, glutamic acid, serine, proline, glycine and alanine were present in relatively high levels. Wang and Fuller (1989) studied the optimum protein composition for pig feed and concluded that the minimum EAA:non-EAA should be at least 45:55 in order to balance the surplus of nitrogen of EAA to be used as a nitrogen source of non-EAA [41]. Since our study showed 49:51 ratio, *S. dimorphus* grown with ammonia gas has potential to be used as an animal feed.

Table 4. 4 Amino acids composition of *Scenedesmus spp.*

AA content (mg/100mg of dry weight)	*S. dimorphus* (present study)	*S. obliquus* (Becker, 1984)	*S. obliquus* (Osman, 2004)
Glutamic Acid	4.19 ± 0.09	5.55	6.01
Aspartic Acid	3.61 ± 0.08	4.36	6.99
Leucine	3.58 ± 0.08	3.79	5.44
Alanine	3.41 ± 0.08	4.67	5.14
Arginine	3.08 ± 0.10	3.68	3.85
Glycine	2.85 ± 0.15	3.68	2.92
Lysine	2.38 ± 0.06	2.91	4.24
Phenylalanine	2.20 ± 0.03	2.49	3.12
Threonine	2.08 ± 0.07	2.65	2.95
Valine	2.17 ± 0.07	3.11	3.91
Proline	2.08 ± 0.03	2.02	3.28
Serine	1.51 ± 0.08	1.97	2.72
Isoleucine	1.55 ± 0.05	1.87	4.97
Tyrosine	1.45 ± 0.03	1.66	2.07
Methionine	0.88 ± 0.03	0.78	1.2
Histidine	0.72 ± 0.02	1.09	1.86
Cysteine	0.53 ± 0.02	0.31	0.08
Taurine	0.03 ± 0.00		
Hydroxylysine	0.09 ± 0.03		
Tryptophan	0.08 ± 0.03	0.16	
Hydroxyproline	0.08 ± 0.02		
Ornithine	0.04 ± 0.01		
Lanthionine	0.00 ± 0.00		
Total AA	38.60	46.74	60.75
Essential AA (EAA)	18.72	22.51	31.54
Non-EAA	19.87	24.23	29.21
EAA/non-EAA	0.94	0.93	1.08
EAA/total AA	0.49	0.48	0.52

The ideal protein recommendation for each animal has not yet been determined and several different proposals are used. Amino acid profile is usually expressed relative to lysine because lysine is the first limiting amino acid in corn, which is the cheapest and most common animal feeds, and it is not used for the synthesis of other nitrogen-compounds [42]. The relatively expressed amino acids of *S. dimorphus* in present study grown at the optimal condition (NH_3 60ppm, pH 7, $0.1day^{-1}$) is compared with published ideal protein recommendations in table 4.5.

Compared to the ideal proteins shown in table 4.5, *S. dimorphus* has relatively balanced amount of methionine, isoleucine, histidine with lysine. However, it contains relatively high amount of threonine, leucine, valine, and arginine and relatively low amount of cysteine and tryptophan. Because this imbalance might impair the quality as an animal feed, further study to find optimal growth condition to produce more idealistic amino acid profile is needed.

Becker (2006) tested nutritional quality of *S. obliquus* and it was comparable to eggs and soybeans [21]. Likewise, nutritional and toxicological evaluations focused on *S. dimorphus* grown with ammonia gas are needed to be fully commercialized as an animal feed.

Table 4. 5 Comparison with ideal protein recommendations

AA profile	*S.dimorphus*	ideal protein for broilers [43]						Ideal protein for dairy cows [44]				ideal protein for growing pigs [42]							
		[45]	[46]	[47]	[48]	[49]	[50]	[51]	[52]	[53]	[54]	[55]	[56]	[57]	[41]	[58]	[59]	[50]	[60]
Lysine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Methionine	37	36	38	38	45	37	44	37	31	35	34								
Met+Cys	59	72	73	70	82	74	79					50	60	55	63	59	60	60	50
Threonine	88	67	65	60	73	65	65	47	75	71	55	60	60	64	72	75	65	64	66
Tryptophan	4	16	16	16	18	16	19	15				15	18	16	18	19	18	19	18
Isoleucine	65	67	66	55	73	67	78	61	71	74	61	55	60	61	60	61	60	54	50
Leucine	151	109		102	109		150	110	123	131	122	100	72	80	110	110	100	100	100
Valine	91	77	80	61	82	75	84	73	81	85	73	70		64	75	75	68	70	70
Histidine	30	32			32		35	36	33	33	42	33	26	29		32	32	36	33
Phenylalanine	93							62	76	72	63								
Phe+Tyr	153											96	100	88	120	122	95	95	100
Arginine	130	105	105	110	114	103	117	43	63	67	43			42			42	31	

Chapter 5. Conclusion

A robust microalgae, *Scenedesmus dimorphus* was used as a model strain to mitigate ammonia gas from animal house. Dilution rate (0.05, 0.1, 0.2, and 0.3 day^{-1}), ammonia gas concentration in the inlet gas (17, 27, 42, 60, and 72 ppm), and medium pH (5, 6, 7, and 8) were tested to find an optimal condition for the algae growth. The algae growth was compared using cell density and cell productivity and turned out that the optimal growth condition is dilution rate 0.1 day^{-1}, ammonia gas concentration in the inlet gas 60 ppm, and medium pH 7.

The ammonia gas removal performance was compared using volumetric ammonia removal capacity, cellular ammonia consumption rate, cell yield, and ammonia gas removal rate. The dilution rate effect was significant in the cell yield: substantially high cell yield was obtained at 0.2 and 0.3 day^{-1}. The ammonia concentration in the inlet gas affected the volumetric ammonia removal capacity and the ammonia gas removal rate. As the concentration increase, the volumetric ammonia removal capacity was increased and the ammonia gas removal rate was lower than 90% at 17ppm. The medium pH became meaningful when comparing the ammonia gas removal rate: when the medium become basic, the ammonia gas removal rate was below 90%.

The nitrogen mass balance was also calculated for all growth conditions. Throughout all cases, the majority of ammonia gas was absorbed by the algae cell. The liquid absorption was affected by the dilution rate the medium pH. Also, the liquid phase ammonia residue seems to have a long-term effect although the cell suspension was continuously exchanged with the fresh medium. The ammonia outlet ventilation was affected by the medium pH: it became significant when the medium was basic (pH 8).

The algae biomass was characterized in terms of amino acid profile. It turns out that the essential amino acid ratio to the non-essential amino acid is high enough to be used as an

animal feed. Therefore, the algae biomass produced with the ammonia gas has a potential as an animal feed. This potential can be realized with further research on its nutritional and toxicological evaluations.

Overall, the concept of producing algae using waste ammonia gas brought a promising result in present study. However, this idea has some limits before full implementation. First, because the algal- ammonia scrubber system is a kind of ‘end-of-pipe’ method, only modern animal houses that are equipped with ventilation pipe can utilize this system. This is not a big obstacle in that most of commercial animal houses are built with ventilation ducts.

Second, the algae growth condition can be diversified to be close to a real animal house situation. For example, the limiting nutrient can be extended to other gases such as CO_2 and CH_4 that are high in animal house outlet gases to see any possible interactive effect between different gases on algae. Also, the light intensity or light-dark period can be interesting parameters.

Finally, scale-up to a pilot scale can give a more realistic result. While the lab scale experiment suggests the possibility, the pilot scale will elucidate more practical problems and its feasibility will be determined.

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